DOCTOR OF MEDICINE

Insulin resistance, chronic heart failure and potential treatment

Wong, Aaron K. F.

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University of Dundee

Insulin Resistance, Chronic Heart Failure And Potential Treatment

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INDEX OF ABBREVIATIONS

CHF	Chronic Heart Failure
HF	Heart Failure
IR	Insulin Resistance
NYHA	New York Heart Association
DM	Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
SD	Standard Deviation
OR	Odd Ratio
CI	Confidence Intervals
LVEF	Left ventricular Ejection Fraction
HbA1c	Glycated Haemoglobin A1c
FIRI	Fasting Insulin Resistance Index
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance
ACEi	Angiotensin Converting Enzymes Inhibitor
TZD	Thiazolidinediones
eGFR	Estimated Glomerular Filtration Rate
ED	Endothelial Dysfunction
CVD	Cardiovascular Disease
NO	Nitric Oxide
SNS	Sympathetic Nervous System
RAS	Renin Angiotensin Aldosterone System
MRA	Minerocorticoids Receptors Antagonist
CPET	Cardiopulmonary Exercise Testing
AMPK	5'-AMP-activated protein kinase
AICAR	5-aminoimidazole-4-carboxamide riboside

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DECLARATION

I declare that this work has not previously been submitted for a higher degree. The design on the work presented in this thesis was that of the author and his supervisors, Professor Chim Lang and Professor Allan D Struthers. The author performed all research works unless acknowledged otherwise. Statistical support was provided by Dr Simon Ogston and Dr Donald Ang.

Ms Lesley McFarlane and Dr Velerie Godfley conducted all the laboratory analyses. The initial data of metformin use and mortality in CHF and glycaemic control in CHF study were collected and analysed by Dr Dougie Elder.

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Aaron KF Wong 2013

THESIS OUTLINE

Diabetes Mellitus (DM) and insulin resistant (IR) are highly prevalent among heart failure (HF) patients. There is now increasing evidence to suggest a bi-directional relationship between IR and HF. DM and IR not only lead to heart failure, but heart failure can also lead toward the development of DM or IR.

The degree of IR also correlates with the severity and mortality of CHF. The pathophysiology of IR in CHF has yet to be fully defined. Activation of sympathetic nervous system, abnormal regulation of adipocytokines systems, activation of inflammatory and coagulation cascade, accumulation of glycated products, endothelial dysfunction and hyperinsulinaemia are potential explanations of the development of IR in CHF. Additionally, it remains to be determined if IR is merely a marker reflecting the severity of CHF or whether it contributes to the disease in CHF. If IR is truly a culprit that worsens CHF, reversing IR may potentially be a new target for treatment in CHF, which may result in an improvement in symptoms and even mortality in patients with CHF. However, there are concerns over the use of certain insulin sensitizers, most notably, the thiazolidinediones (TZDs), which has been linked with increased risk of hospitalizations for CHF and concerns regarding its association with increased myocardial infarction. Despite previous concerns of lactic acidosis, there is now evidence that metformin may not only be safe but could potentially be useful in the setting of CHF. We have conducted a randomised double-blind, placebo-controlled trial testing the hypothesis of reversing IR with metformin in insulin-resistant CHF will have beneficial effects. If IR is a possible target for the treatment of CHF, what are the new and

potential treatment modalities? We have now had better understandings of the adipocytokines systems, which may prove to be a therapeutic option to improve IR in CHF. AMP-activated protein kinase (AMPK) pathway has become the focus of research as a novel therapeutic target in cardio-metabolic disease. It has been shown to mediate, at least in part, the effects of a number of physiological and pharmacological factors that improve IR. It also exerts beneficial effects on the vasculature and the heart. There have been some new AMPK activators that are currently being tested in vivo setting or phase 1-2 trials, and the early results are somewhat promising.

Increased understandings and refreshed insights of IR and CHF have opened a new horizon and encouraged us to explore more therapeutics options in CHF.

CHAPTER 1: DIABETES AND CHF

INTRODUCTION

Diabetes and CHF often co-exist with an inter-relationship such that each condition may impact on each other in terms of causation and outcome (Figure 1). The Framingham Study highlighted the co-existence of diabetes and CHF (1). Kannel et al reported that 19% of patients with CHF in the Framingham Study have diabetes and that the risk of CHF increases by 2-8 folds in the presence of diabetes (1). The prevalence of DM is around 4-7% in the general population and 0.5% of the general population has both DM and HF (2, 3). From population-based studies and in CHF trials, the prevalence of T2DM is estimated to be between 11% and 28% and increased to 25-30% among all patients hospitalized for CHF (4-6) (Table 1).

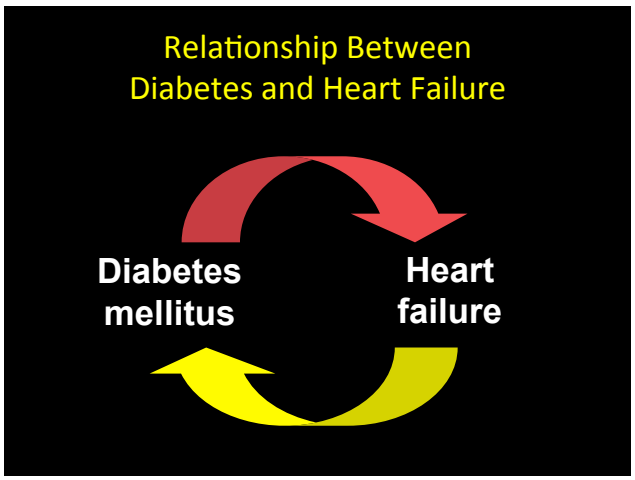

FIGURE 1: Bi-directional relationship between DM and HF

Study and date	Type of study	Number of participants	Age range	Mean age (years)	Definition of LVSD	Prevalence of LVSD	Prevalence of symptomatic/ asymptomatic LVSD	Population with LVSD—prevalence of DM	Population without LVSD—prevalence of DM
ECHOES, England[3] 2001	Epidemiological—primary care	3960	>45	61	LVEF <40%	*n* = 72 (1.8%)	Symptomatic, *n* = 38 (1%), asymptomatic, *n* = 34 (0.8%)	Symptomatic, *n* = 9 (24%), asymptomatic, *n* = 2 (6%)	*n* = 146 (3.8%)
Copenhagen[119] 2003	Epidemiological—primary care	764	50–89	66 (Median)	LVEF ≤40%	*n* = 36 (4.7%)	33% Asymptomatic	*n* = 5 (7.2%)	*n* = 43 (5.9%)
Poole, England[120] 1999	Epidemiological—primary care	817	70–84	76	Qualitative assessment	*n* = 61 (7.5%)	79% Asymptomatic	*n* = 6 (10%)	*n* = 43 (6%)
Glasgow[2] 1997	Epidemiological	1640	25–74	50	LVEF ≤30%, LVEF ≤35%	*n* = 43 (2.9%), *n* = 113 (7.7%)	77% of participants with LVEF ≤35% were asymptomatic	*n* = 14 (12.4%)	*n* = 34 (2.5%)
Vasteras, Sweden[121] 2001	Epidemiological	412	75	75	LVWMI <1.7%	*n* = 28 (6.8%)	46% asymptomatic	*n* = 6 (22%)	*n* = 27 (7%)
Olmsted, USA[122] 2003	Epidemiological	1888	>45	63	LVEF ≤50%, LVEF ≤40%	*n* = 123 (6.5%), *n* = 40 (1.8%)	–	*n* = 21 (17%), *n* = 6 (15%)	*n* = 130 (6.8%)
Copenhagen[123] 2005	Prospective, hospital clinic	188	–	69	LVEF <45%	All had LVSD	All were symptomatic	*n* = 48 (25.5%)	–

LVSD, left ventricular systolic dysfunction; DM, diabetes mellitus; LVEF, left ventricular ejection fraction; LVWMI, left ventricular wall motion index.

TABLE 1: The prevalence of DM in populations with and without LVSD.

MacDonald MR et al. Eur Heart J 2008;29:1224-40

As stated earlier, there has been a pathophysiological linkage between T2DM and HF. There are numbers of independent risk factors that were identified as predictors of the development of HF in DM. These include increased body mass index (BMI), age, the presence of coronary artery disease, New York Heart Association functional class and glycaemic control measured by HbA1c (6-8).

NYHA FUNCTIONAL CLASS – PREDICTOR OF RISK OF DEVELOPING DM IN CHF

In an Italian population-based study of 1,339 elderly subjects with a mean (+/- SD) age of 74.2 +/- 6.4 years. CHF has been shown to be a strong predictor of the development of DM independently of age, sex, family history of diabetes, BMI, waist/hip ratio, systolic and diastolic blood pressure, and treatment for CHF (OR = 1.4, 95% CI = 1.1-1.8)(9). This strongest association was observed in patients with more severe HF (NYHA III and IV) than patients with milder HF (NYHA I and II). Similar observation was found in the Bezafibrate Infarction Prevention study. Incidence of diabetes was determined by baseline NYHA functional classification. 2616 non-diabetic patients aged 45 to 74 years were divided into three groups according to New York Heart Association (NYHA) criteria: class I (n = 1986 patients), class II (n = 518), and class III (n = 112). The detection of a fasting blood glucose level ≥7 mmol/L during follow-up was defined as the criterion for the development of diabetes. 259 patients (13%) in NYHA class I developed DM, 76 (15%) in class II, and 22 (20%) in class III (P for trend = 0.05) during 8 years follow up of this study. NYHA class III were twice as likely (17% [n = 19]) to have fasting blood glucose levels of ≥7 mmol/L than those in NYHA class I (7.8% [n = 154]) or class II (8.7% [n = 45]) (P = 0.005) (Figure 2). In a multivariate analysis, NYHA class III was found to be the strongest predictor of the development of DM associated with a 1.7-fold (95% confidence interval [CI]: 1.1 to 2.6) increase of the rate of

development of diabetes, but not NYHA class II (hazard ratio = 1.0; 95% CI: 0.8 to 1.3) (10).

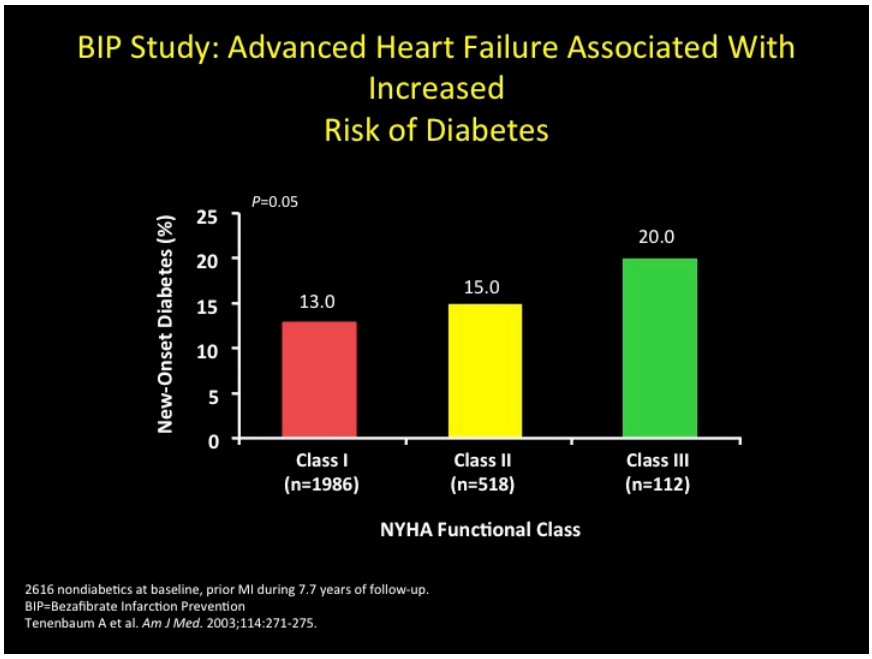

FIGURE 2 Bezafibrate infarction prevention study.

Advanced HF associated with increased risk of DM. Tenenbaum A et al. Am J Med 2003;114:271-275

GLYCAEMIC CONTROL- PREDICTOR OF RISK OF DEVELOPING HF IN DM

The risk of CHF appears to be related to the blood sugar control in patients with diabetes. Iribarren and colleagues demonstrated that a 1% increase in HbA1C was associated with an 8% increased in risk of CHF independent of blood pressure, body mass index, age and presence of coronary artery disease (1). Conversely, the UKPDS study showed that a 1% reduction of HbA1c was associated with a 16% reduced risk of developing CHF (2). The presence of diabetes mellitus is also associated with worse outcome in CHF trials. In the Left Ventricular Dysfunction (SOLVD) trial, diabetes mellitus was an independent predictor of mortality and morbidity in patients in CHF (3). Similarly, in the Beta-Blocker Evaluation of Survival Trial (BEST), patients with DM were associated with more severe HF and adverse outcome compared to CHF patients without DM (4). Held et al (5) showed each millimols per litre increased in fasting plasma glucose in patients with diabetes was associated with a 1.10-fold-increased risk of CHF hospitalization after adjustment for age and sex. All these findings showed a clear and important link between diabetes and CHF.

However, we are not certain of the association between the degree of dysglycaemia and risk of HF. If the degree of dysglycaemia does associate with increased risk of HF, then the next question naturally asked is what is the degree of dysglycaemia carries the highest risk of developing HF in patients with T2DM? We would have as a matter of course thought that more severe

dysglycaemia is associated with a higher incidence of HF than patients with normoglycaemia. However, there have been some controversies regarding the degree of dysglycaemia and incident HF. Recent retrospective studies have achieved different conclusions.

The benefit of improved glycaemic control on microvascular complications in T2DM is well established (7,14), and recent trials have attempted to clarify the role of glycaemic control on macrovascular outcomes (15,16). These data suggested that improved glycaemic control has the potential to reduce the risk of both micro- and macrovascular disease significantly when instigated early in the disease course, but in more advanced T2DM, the benefits of improved control were less evident (17). Furthermore, recent studies suggested that tight glycaemic control can sometimes be associated with a poorer macrovascular outcome (18,19) than standard care. In patients with co-existing CHF and T2DM, the relevance of good glycaemic control is a critical issue not only as the combination may be associated with a significantly poorer outcome but also as the choice of drugs available to manage hyperglycaemia in CHF are perhaps more limited (20,21). There are conflicting reports of the importance of glycaemic control in patients with T2DM and CHF (22-26). The relationship between glycaemic control and outcomes has been reported to be “U” shaped (26), “J” shaped (25), linear (24), and even inverse (23). The main findings from relevant trials regarding incident HF and degree of dysglycaemia are summarised as followed:

UKPDS—HBA1C, A PROGRESSIVE RISK FOR ADVERSE OUTCOME (2)

In year 2000, UKPDS 35 trial examined the relationship between long-term glycaemic control and micro- and macrovascular outcome in patients with Type 2 diabetes. The primary endpoints of the study were diabetes related death and all cause mortality. Secondary endpoints were myocardial infarction, stroke, amputation (including death related to peripheral vascular disease), and microvascular disease (predominantly retinal photo-coagulation). Other outcome measures were incident heart failure and cataract extraction. Adverse outcome was associated with the degree of dysglycaemia. The authors concluded that each per cent of mean HbA1c reduction was associated with 21% reduction of diabetes related endpoint (95% confidence interval 17% to 24%, P<0.0001), 21% for deaths related to diabetes (15% to 27%, P<0.0001), 14% for myocardial infarction (8% to 21%, P<0.0001), and 37% for microvascular complications (33% to 41%, P<0.0001). The risk of HF increased by 16% for each per cent increased of mean HbA1c overtime (p=0.021), adjusted for age of diagnosis of DM, sex, ethnic group, history of smoking, presence of hypertension and microalbuminuria, and dyslipidaemia. No threshold of risk was observed for any end point. The risk of adverse outcome was the lowest in patients with HbA1c within the normal range (<6%) (2).

VETERANS AFFAIRS STUDY—U SHAPE CURVE RELATIONSHIP (6)

At the Veterans Affairs medical centres, Aguilar et al conducted a retrospective study to determine the relationship between HbA1c and CHF by assessing the association of different quintiles of HbA1c and CHF-related outcome (mortality and risk of HF hospitalization). 5815 veterans with CHF and T2DM treated in ambulatory clinics were included in the study. After periods of two years follow up, a U shape curve relationship was found between HbA1c and mortality. Death occurred in 25% of patients in Quintile1 (HbA1C ≤6.4%), 23% in Quintile 2 (6.4% 9.0%). The middle quintile was found to have the lowest mortality after adjustment for potential confounders when compared with the lowest quintile (risk-adjusted hazard ratio: 0.73, 95% confidence interval: 0.61 to 0.88, p = 0.001). Conversely, it was a linear relationship for HF hospitalization with increasing quintiles of HbA1C (Q1: 13.3%, Q2: 13.1%, Q3: 15.5%, Q4: 16.4%, and Q5: 18.2%). However, this association was not statistically significant when adjusted for potential confounders (26)(Figure 3), suggesting that the differences in baseline demographics and treatments may be accounting for the increased rate of heart failure hospitalization This highlighted the complex relationship between HbA1c and mortality in patients with diabetes and heart failure. Patients in the lower and higher quintiles of HbA1c have a higher mortality than patients with modest glycaemic control. They postulated that the increased mortality in patients with the higher quintile of HbA1c levels was likely multifactorial secondary to the direct and indirect effects of hyperglycaemia. The adverse effects of hyperglycaemia include increased oxidative stress, endothelial dysfunction, increased protein kinase C

activation, and ultimately accelerate atherosclerosis. Chronic hyperglycaemia is associated with accumulation of advanced glycation end products, which lead to increased myocardial stiffness, and dys-regulation of various cellular signaling pathways, and eventually cellular dysfunction. Elevated HbA1c is also linked with increased insulin resistance and may also be related to poor compliance with medications, which in turn may be associated with a poor outcome. The potential explanations of increased mortality in patients with the lowest quintile of HbA1c are likely to be secondary to the hazardous effects of intensive glucose control, and possibly related to protein malnutrition and an increased in inflammatory syndrome associated with advanced heart failure.

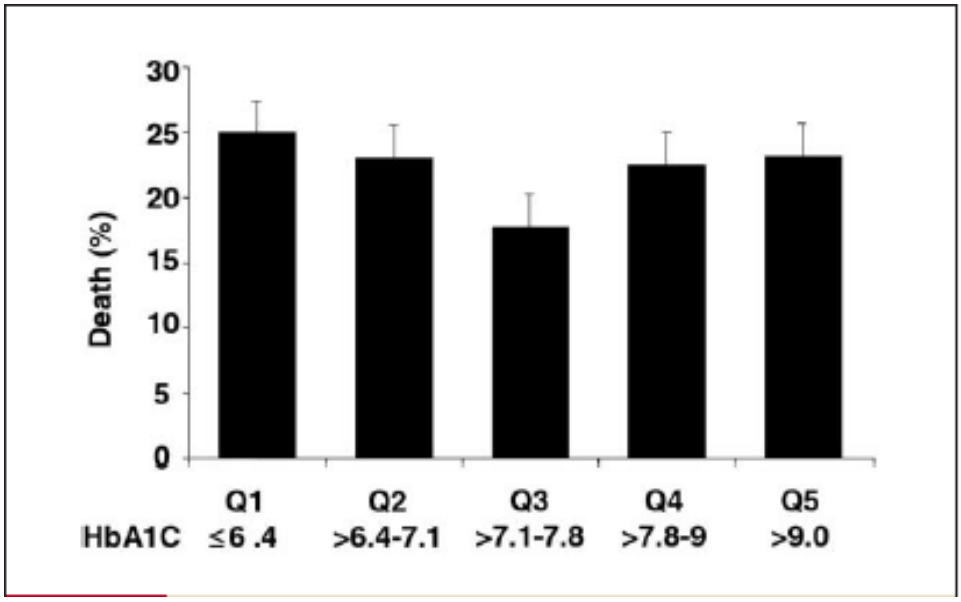

FIGURE 3: The association between mortality and HbA1C in diabetic patients with HF appears U-shaped, with the lowest risk of death in those patients with modest glucose control (7.1% < HbA1C ≤7.8%).

The graph represents the proportion of patients who died at 2-year follow-up with quintiles (Q) of glycosylated haemoglobin (HbA1c). Global chi-square p=0.001. Error bars indicate the 95% confidence intervals. Aguilar et al. J Am Coll Cardiol 2009;54:422-8

ESHAGHIAN AND COLLEAGUES—A PARADOXICAL RELATIONSHIP OF HBA1C AND OUTCOME (7)

The relationship between HbA1c and mortality in patients with advanced HF and T2DM was evaluated by Eshaghian et al. 123 patients with T2DM and advanced HF with HbA1c measured at presentation were included in the study. Patients were then divided into two categories: HbA1c>7% (n=74) and ≤7% (n=49). More than two third of the cohort was men with the mean ejection fraction of 25% +/- 7. Of which, 60% has ischaemic cardiomyopathy with mean HbA1c of 7.9% +/- 1.8, and diabetes duration of 8.6 +/- 9 years. Both groups were matched for age, sex, New York Heart Association class; body mass index; diabetes duration; anti-diabetic medications used. Patients with HbA1c >7.0 were associated with higher ejection fraction, increased β-blocker and sulfonylurea use. Contrary to previous perception of better glycaemic control leads to a better outcome, this study found a paradoxical relationship of HbA1c and adverse outcome. Patients with low HbA1c of ≤7.0 were found to have a significantly increased all-cause mortality, compared with those with HbA1c >7.0 (35% vs. 20%, hazard ratio 2.6, 95% CI 1.3-5.2, P<0.01).

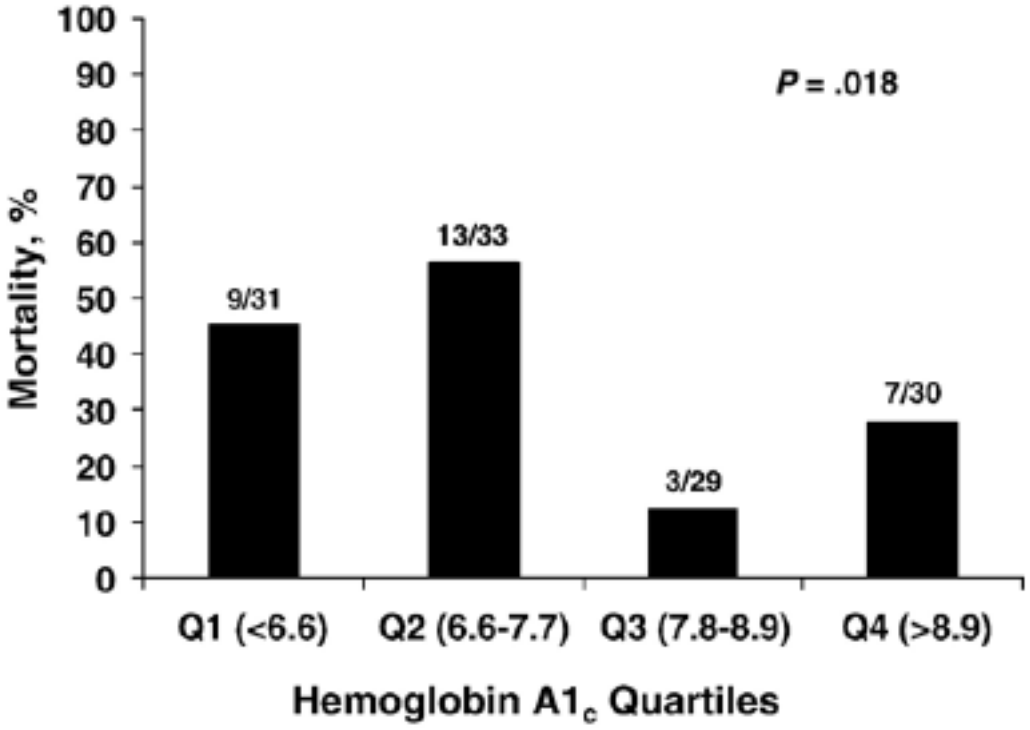

FIGURE 4: A Paradoxical Relationship of HbA1c and outcome.

Paradoxically, elevated HbA1c levels were associated with improved survival in this cohort of patients with diabetes and advanced HF. Mortality rates by Kaplan-Meier analysis for advanced systolic heart failure patients with diabetes at 2 years by HbA1c quartiles. The number of events and the number of subjects in each quartile are shown above each bar. Eshaghian et al. Am Heart J 2006;151:91

WHAT DO WE LEARN FROM CHARM? —LINEAR RELATIONSHIP BETWEEN HBA1C AND ADVERSE OUTCOME (8)

From the Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program, we learned that diabetes is a progressive risk. The CHARM study followed up 2412 patients (907 with a previous history of diabetes) with at least one HbA1c measurement for a median of 34 months. The primary outcome consisted of CV death, HF hospitalization, and total mortality was determined. Almost all the patients were followed up until they have developed outcome or until the study finished. Authors have found a linear relationship between HbA1c and risk of adverse events. After adjusted for sex and age, hazards ratios of these outcomes per 1% higher HbA1c level were 1.25 (95% confidence interval [CI], 1.20-1.31) for CV death, 1.24 (95% CI, 1.17-1.31) for HF hospitalization, and 1.22 (95% CI, 1.16-1.29) for total mortality. This relationship remained evident in patients with and without diabetes, with reduced or preserved ejection fraction and persisted after adjustment for diabetes, other risk factors, and allocation to preserved EF or low EF (Figure 5).

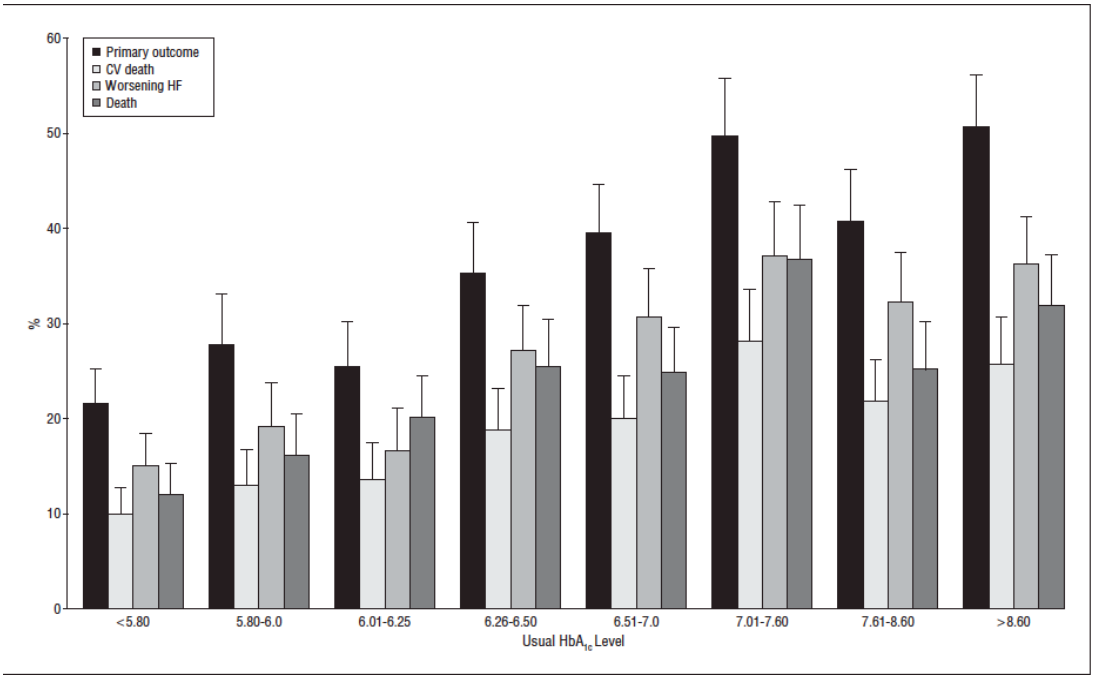

FIGURE 5: Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program.

In diabetic and non-diabetic patients with symptomatic chronic HF, HbA1c level is an independent progressive risk factor for CV death, hospitalization for HF, and total mortality. (P for trend <0.001). Error bars indicate 95% confidence intervals. Gerstein HC et al. Arch Intern Med 2008;168:1699-704

ATHEROSCLEROSIS RISK IN COMMUNITIES (ARIC) STUDY-INCREASED HBA1C ASSOCIATE WITH INCREASED RISK OF DEVELOPING HF (9)

In a prospective population-based study, Pazin-Filho et al studied the incidence of death and HF hospitalization in patients with diabetes but no evidence of HF at baseline. Each per cent increase of HbA1c was associated with 17% increased risk of developing HF in the non-coronary heart disease group. The risk was 20% in patients with coronary heart disease. HbA1c has been shown to be an independent predictor of risk of developing HF in diabetic patients with and without coronary heart disease(9).

SWEDISH NATIONAL DIABETES REGISTRY STUDY- POOR GLYCAEMIC CONTROL WITH HBA1C>7% ASSOCIATED WITH INCREASED RISK OF HOSPITALISATION OF HF

In a Swedish National Diabetes Registry study, Lind et al has identified 10,969 (13.2%) among 83,021 patients with T2DM who were hospitalized with a primary or secondary diagnosis of heart failure during a mean follow-up of 7.2 years. Male sex ($p < 0.001$), older age ($p < 0.001$) and longer diabetes duration ($p < 0.001$) correlates with increased incident HF hospitalization. After adjusting for risk factors of heart failure, the HR per each percentage unit higher HbA_{1c} for heart-failure hospitalization was 1.12 (95% CI 1.10, 1.14). By category of mean HbA_{1c}, the HR for heart failure hospitalization was: HbA_{1c} 6.0 to <7.0%, 0.91 (95% CI 0.84, 0.98); HbA_{1c} 7.0 to <8.0%, 0.99 (95% CI 0.91, 1.07); HbA_{1c} 8.0 to <9.0%, 1.10 (95% CI 1.01, 1.20); HbA_{1c} 9.0 to <10.0%, 1.27

(95% CI 1.15, 1.41); HbA_{1c} ≥10.0 %, 1.71 (1.51, 1.93) (reference HbA_{1c} <6%). The HR for patients with HbA_{1c} 7.0 to <8.0% compared with patients with HbA_{1c} 6.0 to <7.0% was 1.09 (95% CI 1.03, 1.14). They concluded that HbA_{1c} >7% is associated with an increased risk of heart failure hospitalization in patients with T2DM (10).

CHAPTER SUMMARY

The bi-directional relationship between DM/IR and CHF is well established. NYHA functional class is the strongest predictor of the development of DM in CHF, whereas glycaemic control is the strongest predictor of the development of CHF in DM. The level of glycosylated haemoglobin (HbA1c) provides a measure of the glycaemic control of patients with T2DM during the previous 2–3 months (11). It is a useful prognosticator in patients with DM and CHF as demonstrated from the above studies. However, we are not certain about what is the optimal glycaemic control in patients with DM and CHF. Studies that assessed the importance of glycaemic control in diabetic patients with CHF (6-9,12) usually used a single measure of HbA1c which underestimated the importance of overall glycaemic control (13). Calculation of a mean HbA1c has been found to be a better predictor of diabetic complications (2,14,15) as it incorporates multiple measures over time (2,13). Therefore, we conducted a study to determine the relationship between *mean HbA1c* and outcome in a large cohort of patients with T2DM and incident CHF.

CHAPTER 2: GLYCAEMIC CONTROL AND OUTCOME IN PATIENTS WITH CHF

STUDY AIM AND OBJECTIVE

In a retrospective analysis, we sought to determine the relationship between mean HbA1c and outcome in a large cohort of patients with T2DM and incident CHF.

STUDY POPULATION AND DESIGN

We performed a retrospective cohort study within the population of Tayside, Scotland (population 400,000) between 1st January 1992 and 31st March 2010 exploiting the unique advanced medical informatics infrastructure available in the region. This makes use of a unique health record identifier – The Community Health Index number (CHI) which has been used for all patient healthcare activity in the region for the past 20 years. It means multiple clinical data sets can be deterministically linked at the level of the individual with high accuracy. Study subjects had both T2DM and CHF and were anonymously identified from three data resources; the Diabetes Audit and Research in Tayside Study (DARTS) (16), the Tayside echocardiographic database (>100,000) maintained by the Department of Cardiology, Ninewells Hospital and the Health Informatics dispensed prescribing database developed by the Medicines Monitoring Unit (MEMO)(17), which holds details on all dispensed prescriptions for all individuals in the region since 1993.

CHF was defined as a record of an echocardiogram with evidence of left ventricular systolic dysfunction (LVSD) and either a prescription for a loop-diuretic (provided not greater than 1 year prior to echocardiogram) or an admission to hospital with an associated heart failure diagnostic code (ICD-9 428, ICD-10 I50). The index date for development of CHF was defined as the minimum of first echocardiogram, valid prescription for a loop diuretic or admission to hospital with CHF. We previously used similar criteria to identify CHF from large datasets in Tayside, and have refined the criteria for CHF with the inclusion of echocardiographic information (18).

HbA1c Measures

To be included in the study, patients were required to have at least two HbA1c measures recorded between index date and end of study. HbA1c was analysed as an updated mean. The updated mean was calculated for each individual from each year of follow-up e.g. in year 1 the mean of the baseline HbA1c and all other HbA1cs measured in the first 12 months was calculated, year 2 is the mean of all measures at baseline years 1 and 2 and so forth until the end of the study period. A weighted mean HbA1c was calculated using all available HbA1c measures during the 'at risk' study time. The mean was weighted by time between measures and was then used to group patients into five categories of HbA1c (≤6%, >6-≤7%, >7-≤8%, >8-≤9% and >9%).

STATISTICAL ANALYSIS

Cox's proportional hazards models (19) were used to model time to all cause death. The entry date was index date for diagnosis with CHF. Updated mean HbA1c was analysed as a time-dependent covariate. Other covariates, averaged over the study period, which were utilized as continuous variables in the model included: age at diabetes diagnosis, age at index date, and estimated glomerular filtration rate (eGFR). History of smoking, history of ischaemic heart disease and cardiovascular medication use (aspirin, statins, thiazide diuretics, beta blockers, ACE inhibitors or ARBs, calcium channel blockers) were included as dichotomous variables. Diabetic medications (insulin therapy, oral hypoglycaemics only or no drug therapy) were considered as dichotomous time-dependent covariates. Differences in patient characteristics were determined by chi-squared test for linear trend for categorical variables and ANOVA test for linear trend for continuous variables with a two sided p-value of < 0.05 considered significant. All statistical analysis was performed using SPSS for windows (v9.2)

RESULTS

PATIENT CHARACTERISTICS

From an initial 2567 T2DM subjects in the echocardiographic database with evidence of left ventricular systolic dysfunction, 1597 (62%) had a hospitalisation for CHF and/or valid loop diuretic prescription. After exclusion for CHF incident date preceding DM diagnosis, 1100 subjects were left, of those

795 had an HbA1c measurement during their observable study period (Figure 6).

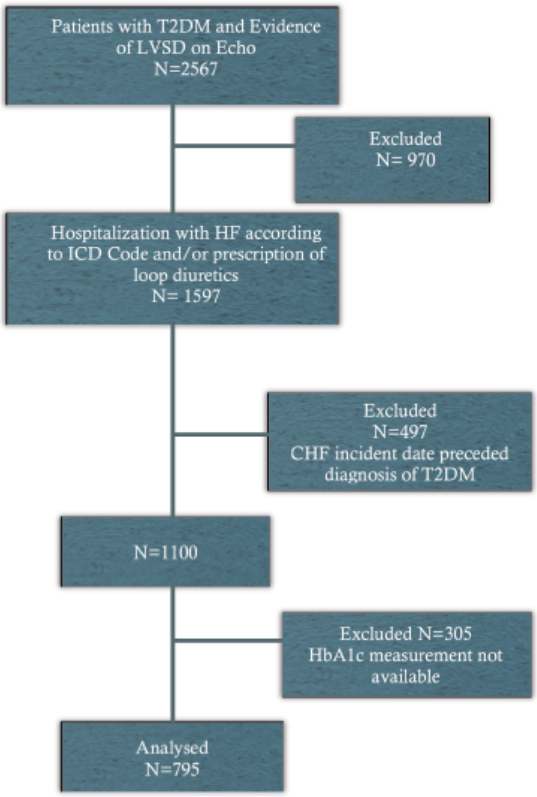

FIGURE 6 CONSORT (Consolidated Standards of reporting trial) Diagram for Glycaemic Control and outcome in patients with CHF

Characteristics of the 795 patients in the study population are provided in Table 2 split by HbA1c category. Patients in the lowest two HbA1c categories had shorter study duration and therefore fewer HbA1c measures. They were diagnosed with CHF and T2DM at an older age, and had a lower BMI and eGFR at baseline. In addition, although not statistically significant, they tended to be more likely to smoke but had fewer myocardial infarction (MI) events prior to baseline. With respect to prescribing, there were relatively more diet treated

and fewer insulin treated patients. They were also more likely to be prescribed thiazide diuretics at baseline. In addition, patients in the lowest category were prescribed less aspirin at baseline. In contrast, patients in the highest HbA1c category were diagnosed with CHF and T2DM at a younger age, had a higher BMI and eGFR, relatively more MI events at baseline, and comprised the smallest proportion of diet and largest proportion of insulin treated patients. In addition, they were more likely to be prescribed aspirin at baseline.

HbA1c and Mortality

Over a median follow up of 3.8 years, there were 491(61.8%) all-cause deaths. In a Cox regression model, adjusted for all other significant predictors, with the middle HbA1c category (>7-≤8%) as the reference, we found a U shaped relationship of HbA1c and outcome with the two lowest and the highest HbA1c categories significantly associated with a higher risk of death (HR 95% CI 1.78(1.26-2.52); 1.29(1.01-1.66) and 1.38(1.03-1.84) respectively) (Figure 7).

HbA1c and Mortality: Diet and drug treated T2DM

To more carefully explore this U shaped association, we considered the HbA1c ≤7% group and made a comparison of patients split by diet and drug treatment (Table 3). The diet treated T2DM patient group were diagnosed with diabetes at an older age, had a lower study and baseline HbA1c and were prescribed less ACE inhibitors at baseline. Significantly, when comparing

baseline HbA1c and study HbA1c, there was no difference in the diet treated group ((mean ±SD) (6.01± 0.64 vs. 6.07± 0.52), p=0.29), but in the drug treated group HbA1c was significantly lower after CHF diagnosis ((7.42± 1.18) vs. (6.36± 0.47), p<0.0001) indicating that drug treatment resulted in more aggressive HbA1c lowering.

We therefore went on to split the entire study population into diet and drug treated to investigate the relationship between HbA1c and death in these groups separately. As the number of patients in each group is smaller and Figure 6 indicated a U-shaped relationship between HbA1c and death, we reduced the number of HbA1c categories to three (≤7, >7-≤9, >9). The adjusted and unadjusted Cox regression models are presented in Table 4. In the diet treated group, lower HbA1c was associated with lower risk of death. Whereas the U shaped association observed in the overall study population remained in the drug treated group.

DISCUSSION

This study had 2 main findings. Firstly, in our cohort of T2DM patients with incident CHF, we observed a U shaped relationship between mortality and glycaemic control, as assessed by a mean HbA1c. Secondly, additional analysis show that this U shape relationship is present in drug treated but not in diet treated T2DM patients. In diet treated patients, lower HbA1c was associated with lower mortality risk. These latter observations may suggest that the outcomes observed in the low HbA1c categories may be related to the response of patients to the DM drug medications.

The relationship between glycaemic control and outcome in patients with CHF and T2DM has previously been studied in at least 4 retrospective studies with different conclusions reported. The relationship between glycaemic control and outcome has been reported to be "U" shaped (6), "J" shaped (12), linear (8) and even inverse (7). In the most recent analysis, Aguilar *et al* (6) performed a retrospective analysis of 5815 veterans (94 % male) with T2DM and CHF defined by clinic coding, 45.5% of which had significantly impaired LV function. Over a 2 year follow-up they observed a U shaped relationship between HbA1c and mortality, with a "sweet spot" seen with individuals in quintile 3 (HbA1c 7.1-7.8 %). Compared to Q3, all other quintiles had significantly elevated risk of death at 2 years with those in the lowest and highest quintiles faring worst. Our data would support these findings. It should be noted that Aguilar's study like all the previous studies, only a single HbA1c was used to assess glycaemic control. However, a single HbA1c may not be reliable, especially if sampled at the time of the diagnosis of CHF when it is potentially influenced by recent alterations in therapy. Individuals may consult physicians with symptoms prior to diagnosis leading to alterations in oral hypoglycaemics or initiation of diuretic therapy that may affect the single HbA1c measurement recorded in the specialist clinic at the time of CHF diagnosis. Indeed, the practice of using baseline HbA1c in studies on diabetes complications can lead to underestimation of the importance of HbA1c as a risk factor, as only one value is used (13,15). Studies have shown that HbA1c levels have a persistent effect on complications several years after their measurement (20,21). Our data are unique as we were able to utilise all HbA1c measures recorded for each individual, enabling us to consider the

importance of longer term glycaemic control over a long period of time in a large patient cohort. In our study, we used a weighted mean to examine the impact of glycaemic control on outcome. The mean HbA1c has been shown to offer superior predictive power over time when compared to a single baseline measure, which can result in underestimations of the impact of glycaemic control (2,13,22). It should be noted that other HbA1c variables have been studied including the last HbA1c value and HbA1c variation as described by standard deviation, neither of which has been shown to be superior to the mean (14,23). Importantly, the weighted mean HbA1c and our median follow-up of 3.8 years enhances the ability of this study to accurately determine the relationship between HbA1c and mortality, as the predictive power of mean HbA1c, out with CHF, is known to increase with longer study length (20-22).

The finding of a higher mortality risk in patients in the lower HbA1c categories (HbA1c ≤6% and HBA1c >6-≤7%) deserves some consideration. In our study, patients in these low HbA1c categories had both favourable and less favourable clinical characteristics. On one hand, these patients had fewer previous MIs and had less intensive DM treatment with less use of insulin. On the other hand, these patients were older when they developed their CHF and they had a lower eGFR. Interpretation of these findings is always going to be limited by a lack of information on the underlying cause of death. However, our finding that this U shaped relationship was present in drug treated but not in diet treated T2DM patients may suggest that the outcomes observed in the low HbA1c categories may be related to the response of patients to the DM medications. This group of patients had developed their CHF later in life and

may be more vulnerable to hypoglycaemia as a result of sulphonylurea and insulin use, which can contribute to morbidity and mortality in high-risk patients with low HbA1c (24). It should be noted that the current findings are concordant with recent ACCORD(25) study, which demonstrated that very tight control of glucose in patients with diabetes may not be beneficial in patients with existing cardiovascular disease and a longer duration of diabetes.

In our study, we also observed a poor outcome in CHF patients with the highest HbA1c. In a sense, this was not unexpected. These patients had more previous MIs at baseline, had more aggressive DM therapy with the largest proportion of insulin treated patients. With respect to CHF patients, there is conflicting data on the relationship between insulin use and outcome in T2DM patients with CHF. Although T2DM on insulin had a higher risk of death in CHF trials (8) (26), the UKPDS 33 study (27) as well as a retrospective cohort study, of 16 000 Medicare diabetic beneficiaries with CHF, showed that insulin use did not predict mortality (28). Those with poorer control also tended to come from more deprived socio-economic groups which is known to be an independent risk factor for poor outcome in diabetes (29). Furthermore, our finding of a poor outcome among patients with poor glycaemic control is concordant with studies showing the wide spread and detrimental effects of hyperglycaemia including progressive atherosclerosis, elevated levels of advanced glycation end products which may lead to increased myocardial stiffness (30), diabetic nephropathy, endothelial dysfunction (31), microangiopathy (32), increased oxidative stress (33) and protein kinase C activation (34).

Obviously, the mechanisms for reduced survival associated with both very tight glycaemic as well those with poor glycaemic control in CHF must remain speculative and cannot be inferred directly from this study.

LIMITATIONS

We recognize the limitations of our study, which are inherent with any retrospective, non-randomized, observational data. However, the current study reflects the true population and a "real world scenario" and adds to previous studies by selecting a large number of patients with T2DM and CHF with a long follow-up period. In common with all observational studies, it was impossible in our study to account for all confounding influences that may have biased the observed differences between the groups considered. We have sought to minimise these as far as practicable by utilising multivariate models and incorporating data on drug prescribing, laboratory blood tests and smoking status. Additionally we utilised multiple HbA1c measures for each individual, and as these were not sampled at specified intervals this may potentially result in survival bias for those who have a greater number of measures, in turn this was minimised by utilising a mean weighted for time. Due to the incidence of recording of renal function and BMI, we utilised a mean value throughout the study period in our model. The study does have much strength including the large number of subjects, the large number of HbA1c measures available, the high event rate (62% died) and the reliable and comprehensive data, which were available with which to build the statistical model.

CONCLUSION

In patients with T2DM and CHF, our observational study shows that there is a U shaped relationship between HbA1c and mortality with the lowest mortality risk in patients with modest glycaemic control (HbA1c, >7-≤9%). This observational data adds support to the growing concern that we need to redefine the optimal HbA1c level in this high-risk group of patients with co-existing T2DM and CHF.

Table 2 Clinical characteristics by HbA1c category

	ALL	HbA1c Category (%)					*P* *
		≤6	>6 - ≤7	>7 - ≤8	>8 - ≤9	>9	
Total number of subjects	795	81(10.2)	232(29.2)	210(26.4)	158(19.9)	114(14.3)	
Follow-up time (years)	3.8(2.0-6.8)	2.7(1.6-6.0)	3.3(1.7-6.1)	4.6(2.4-7.4)	4.4(2.2-7.2)	3.8(2.0-6.4)	**0.0006a**
Time at risk (years)	2.5(1.0-4.8)	1.5(0.7-2.6)	2.1(0.7-4.2)	2.9(1.1-5.4)	2.9(1.2-5.7)	2.6(1.2-4.4)	**<0.0001a**
Time from baseline to study entry (years)	0.6(0.2-1.5)	0.7(0.3-1.5)	0.5(0.2-1.4)	0.5(0.2-1.5)	0.5(0.3-1.6)	0.6(0.3-1.9)	0.6253a
Dead	491(61.8)	50(61.7)	143(61.6)	125(59.5)	97(61.4)	76(66.7)	0.8057b
Males	489(61.5)	49(60.5)	136(58.6)	137(65.2)	94(59.5)	73(64.0)	0.6154b
Age at diabetes diagnosis (years)	62.7(11.4)	65.2(10.7)	67.0(10.4)	61.7(11.5)	59.5(11.6)	58.3(10.4)	**<0.0001c**
Social deprivation:							0.7895b
1 (most)	217(27.7)	24(30.0)	51(22.3)	62(30.0)	47(30.3)	33(29.2)	
2	168(21.4)	13(16.3)	56(24.5)	39(18.8)	35(22.6)	25(22.1)	
3	158(20.2)	21(26.3)	47(20.5)	37(17.9)	28(18.1)	25(22.1)	
4	127(16.2)	13(16.3)	37(16.2)	37(17.9)	24(15.5)	16(14.2)	
5 (least)	114(14.5)	9(11.3)	38(16.6)	32(15.5)	21(13.6)	14(12.4)	
Mean study HbA1c (%)	7.53(1.28)	5.60(0.30)	6.54(0.28)	7.48(0.28)	8.46(0.28)	9.72(0.59)	**<0.0001c**
Number of study HbA1c measures	6 (3-14)	3(1-6)	5(2-10)	8(3-17)	8(4-16)	8.5(3-14)	**<0.0001a**
Baseline Characteristics:							
Age (years)	71.8(9.6)	73.6(9.5)	74.1(9.5)	71.3(9.2)	70.4(9.2)	68.5(9.8)	**<0.0001c**
Mean HbA1c (%)	7.70(1.30)	6.64(1.29)	7.28(1.17)	7.81(1.19)	8.15(1.24)	8.41(1.08)	**<0.0001c**
Ever smoked	450(56.6)	50(61.7)	139(59.9)	124(59.1)	75(47.5)	63(54.4)	0.0913b
MI	408(51.3)	38(46.9)	113(48.7)	104(49.5)	80(50.6)	73(64.0)	0.0626b
MAP (mmHg)	122.5(11.7)	123.8(11.1)	123.0(12.6)	122.4(11.8)	122.2(11.1)	121.1(10.9)	0.5761c
BMI(kg/m^2)	29.6(5.5)	29.1(6.7)	29.0(5.3)	29.5(5.3)	29.9(5.2)	31.0(5.4)	**0.0364c**
eGFR(mmol/L)	61.4(19.3)	57.2(20.3)	59.8(19.7)	61.9(18.4)	62.1(19.1)	65.5(19.1)	**0.0324c**
Diabetes drugs:							**<0.0001b**
Diet	202(25.4)	41(50.6)	79(34.1)	43(20.5)	25(15.8)	14(12.3)	
Oral: No Sulphonylurea	114(14.3)	16(19.8)	39(16.8)	28(13.3)	17(10.8)	14(12.3)	
Oral: Sulphonylurea	321(40.4)	13(16.1)	92(39.7)	97(46.2)	70(44.3)	49(43.0)	
Insulin	158(19.9)	11(13.6)	22(9.5)	42(20.0)	46(29.1)	37(32.5)	
Cardiovascular drugs:							
Statins	332(41.8)	27(33.3)	99(42.7)	89(42.4)	68(43.0)	49(43.0)	0.6173b
ACE	356(44.8)	30(37.0)	105(45.3)	96(45.7)	68(43.0)	57(50.0)	0.4764b
Aspirin	428(53.8)	33(40.7)	131(56.5)	113(53.8)	79(50.0)	72(63.2)	**0.0249b**
Beta Blockers	254(32.0)	25(30.9)	82(35.3)	68(32.4)	41(26.0)	38(33.3)	0.4048b
Thiazide Diuretics	108(13.6)	15(18.5)	43(18.5)	27(12.9)	12(7.6)	11(9.7)	**0.0115b**
Rate limiting CCB	102(12.8)	11(13.6)	24(10.3)	32(15.2)	20(12.7)	15(13.2)	0.6580b
Other CCB	225(28.3)	27(33.3)	58(25.0)	61(29.1)	50(31.7)	29(25.4)	0.4561b

Data are mean (standard deviation), median (interquartile range) or n(%).a Kruskal-Wallis test. b chi-square test. c ANOVA. Bold values indicate a statistically significant test with P<0.05. Frequency missing: social deprivation 11, baseline HbA1c 71, MAP 78, BMI 39, eGFR 30.

Figure 7: Hazard ratio by different HbA1c categories

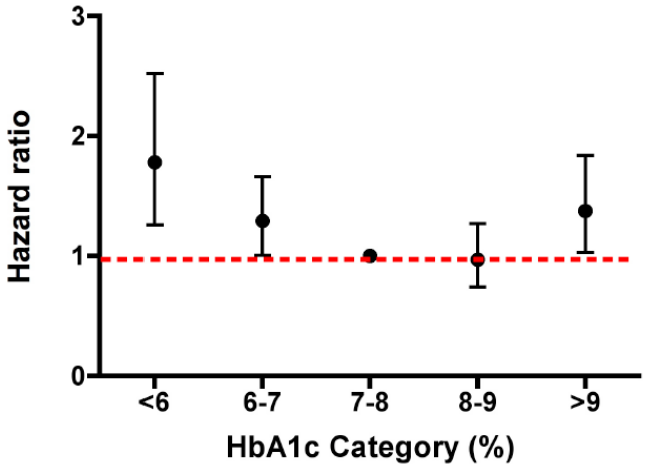

Table 3: Clinical characteristics of HbA1c split by diabetes treatment

	Diabetes treatment		
	Diet only	Drug	*P**
Total number of subjects	74(23.6)	239(76.4)	
Follow-up time (years)	3.4(2.0-6.7)	3.9(1.6-5.7)	0.1758a
Time at risk (years)	2.1(0.8-4.8)	1.8(0.7-3.8)	0.1438a
Time from baseline to study entry (years)	0.5(0.2-1.5)	0.6(0.3-1.4)	0.2639a
Dead	40(54.1)	153(64.0)	0.1235b
Males	42(56.8)	143(59.8)	0.6381b
Age at diabetes diagnosis (years)	70.5(10.8)	65.3(10.0)	**0.0001c**
Social deprivation:			0.8358b
1 (most)	17(23.0)	58(24.7)	
2	18(24.3)	51(21.7)	
3	19(25.7)	49(20.9)	
4	10(13.5)	40(17.0)	
5 (least)	10(13.5)	37(15.7)	
Mean study HbA1c (%)	6.07(0.52)	6.36(0.47)	**<0.0001c**
Number of study HbA1c measures	4(2-8)	4(2-10)	0.1485a
Baseline Characteristics:			
Age (years)	75.7(9.2)	73.4(9.5)	0.0591c
Mean HbA1c (%)	6.01(0.64)	7.42(1.18)	**<0.0001c**
Ever smoked	51(68.9)	138(57.7)	0.0858b
MI	35(47.3)	116(48.5)	0.8522b
MAP (mmHg)	124.1(11.7)	122.9(12.4)	0.5241c
BMI(kg/m^2)	28.1(5.9)	29.3(5.6)	0.1242c
eGFR(mmol/L)	58.7(17.3)	59.2(20.6)	0.8289c
Cardiovascular drugs:			
Statins	30(40.5)	96(40.2)	0.9544b
ACE	24(32.4)	111(46.4)	**0.0335b**
Aspirin	33(44.6)	131(54.8)	0.1241b
Beta Blockers	24(32.4)	83(34.7)	0.7160b
Thiazide Diuretics	11(14.9)	47(19.7)	0.3530b
Rate limiting CCB	11(14.9)	24(10.0)	0.2500b
Other CCB	18(24.3)	67(28.0)	0.5307b

Data are mean (standard deviation), median (interquartile range) or n(%).a Mann-Whitney test. b chi-square test. c t-test. Bold values indicate a statistically significant test with P<0.05. Frequency missing: social deprivation 4, baseline HbA1c 35, MAP 43, BMI 19, eGFR 14.

TABLE 4: Cox models analysing HbA1c by 3 categories

	All (n=795)		Diet only (n=86)		Drug treated only (n=709)	
HbA1c group (%)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI)	Unadjusted HR (95% CI)
≤7	**1.34 (1.09-1.66)**	**1.43 (1.18-1.73)**	**0.17 (0.07-0.39)**	**0.28 (0.14-0.57)**	**1.46 (1.18-1.82)**	**1.64 (1.33-2.03)**
>7 - ≤9	1.00	1.00	1.00	1.00	1.00	1.00
>9	**1.31 (1.00-1.70)**	1.25 (0.96-1.62)	*	*	**1.36 (1.04-1.77)**	1.29 (0.99-1.68)

HbA1c >7 - ≤9% is the reference category. Bold values indicate a statistically significant test with P<0.05. *There were no individuals in this group

CHAPTER 3: INSULIN RESISTANCE AND CHF

INTRODUCTION

Diabetes and chronic heart failure (CHF) often co-exist with an inter-relationship such that each condition may impact on each other in terms of causation and outcome. This bi-directional inter-relationship between CHF and diabetes also extends to insulin resistance (IR). Longitudinal epidemiological data such as the Uppsala study of 1187 middle-aged and elderly men showed that IR precedes and predicts the development of CHF, independent of established risk factors for CHF, including diabetes itself. They found a striking inverse relationship between the risk of incident CHF and insulin sensitivity. Insulin sensitivity was defined by euglycaemic insulin clamp glucose disposal rate (49). Other groups have also investigated the relationship between IR and CHF. Swan et al has previously assessed insulin sensitivity in patients with CHF using minimal modeling analysis (HOMA-IR) during a weight adjusted intravenous glucose tolerance test in which they have established the relationship between the degree of IR and severity of CHF (Figure 8) (50). Patients with CHF were associated with marked IR. Insulin sensitivity was reduced by 58% in patients with CHF compared with healthy control subjects. Moreover, the degree of IR also correlates positively with severity of heart failure characterized by low peak oxygen consumption (VO2). Peak VO2 derived from cardiac pulmonary exercise testing is a useful prognosticator to assess outcomes in CHF (51,52). Doehner et al has shown that the presence and the

severity of IR in patients with CHF were independently associated with a poorer outcome, characterized by reduced peak VO2 and NYHA classes (53).

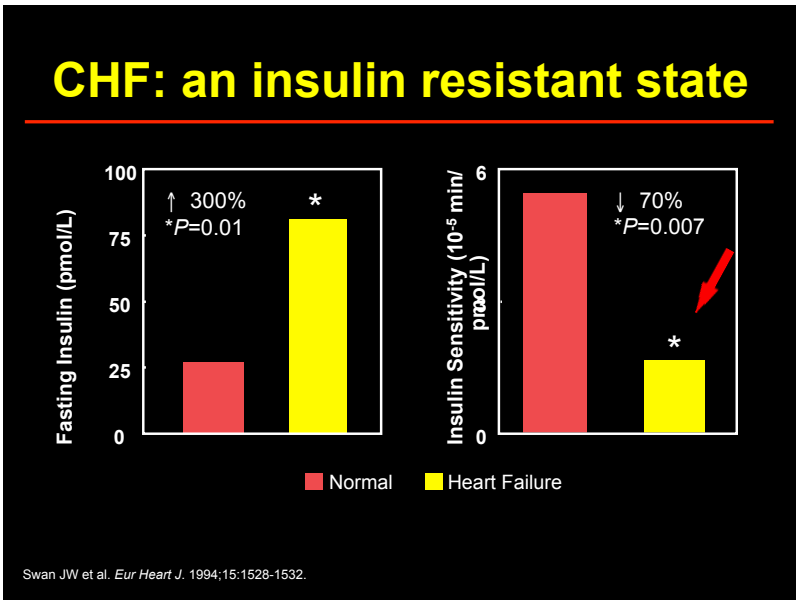

Figure 8: Heart Failure: An Insulin Resistant state

Heart Failure is associated with dysglycaemia and inversely correlates with insulin sensitivity. Swan et al. J Am Coll Cardiol 1997;30:527-32

It should be noted that all these studies have utilized the HOMA-IR during an intravenous glucose tolerance test to determine insulin resistance/ sensitivity, which is a demanding technique. We recently evaluated the prevalence of IR in patients with CHF using fasting insulin resistance index (FIRI), which consists of the product of plasma insulin and glucose divided by 25. The presence of IR is defined by FIRI of ≥2.7 according to local laboratory normal ranges (54). Among 92 non-diabetic patients with CHF, 67% of these patients were insulin resistant. IR is highly prevalent in CHF population, and the degree of IR also correlates with severity of CHF, characterized by higher NYHA classes and lower peak VO2 and cardiac output (55) (Figure 9). The presence of

IR also correlates with poorer endothelial function compared to CHF patients without IR.

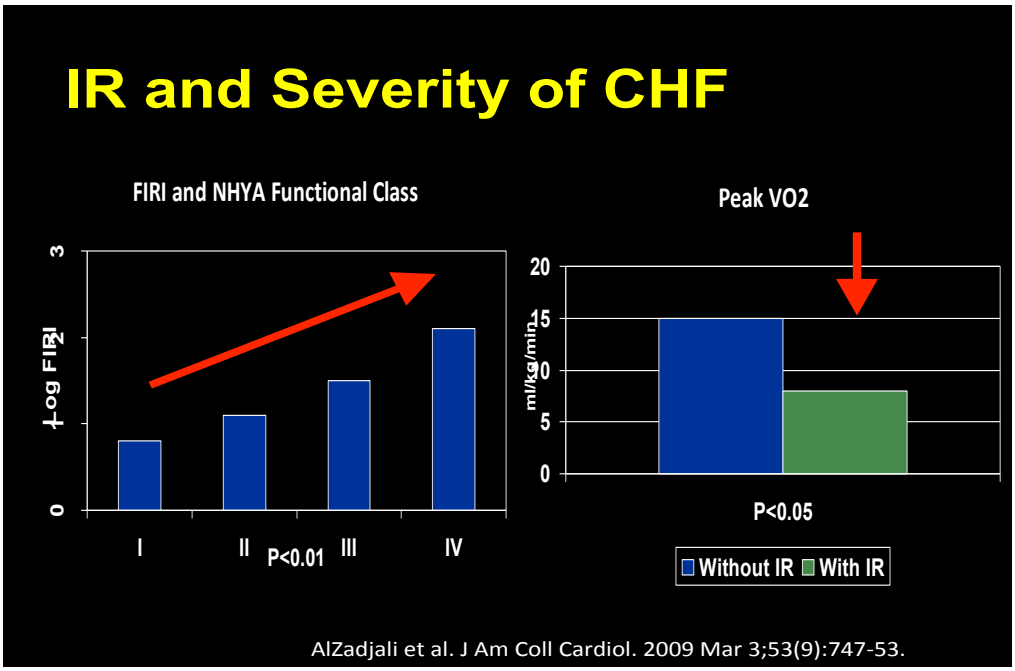

Figure 9: Relationship between Insulin Resistance and Severity of CHF

Degree of insulin resistance correlates with severity of heart failure and insulin resistance is associated with significant reduction in exercise capacity. Alzadjali et al. J Am Coll Cardiol 2009;53:747-53

More importantly, IR in CHF not only predicts disease severity but also mortality (35). In a community-based HF clinic, Goode et al assessed the prognostication of HbA1c for mortality in patients with HF in 970 non-diabetic patients who were referred to HF clinic. Patients with reduced EF of ≤ 45%, HbA1c of >6.7% were associated with an abrupt increase in mortality (n=68) compared with those with HbA1c of ≤6.7% (n = 368) (hazard ratio (HR): 2.4, p<0.001). This observation persisted after adjustment for age and comorbidity (HR 1.9, p = 0.008) with respective 1-year mortalities of 26.5% and 9.4%. Conversely, this increase in mortality was not observed in those with LVEF of

greater than 45% (HR 1.44, p = 0.36 after adjustment)(12). The exact role of IR in patients with CHF is still yet to be defined, as these observational studies do not distinguish between the cause and effect.

PATHOPHYSIOLOGY OF IR AND CHF

Does IR lead to CHF or CHF lead to IR? There are still no definite answers to these questions. IR and CHF are a pair of intricate disease with overlapping pathophysiological processes. What we do know from observational studies on certain rare genetic diseases associated with severe IR such as Alstrom syndrome, 60% of these patients progressed to severe dilated cardiomyopathy (DCM) (56). The presence of cardiomyopathy in the absence of coronary artery disease and hypertension is increasingly recognized in diabetic patients, but the exact pathophysiology is still remained to be defined. Rodent models were used to study the relationship between diabetes and cardiomyopathy as rodents are resistant to atherosclerosis; therefore, these have provided evidence on the occurrence of IR or diabetic cardiomyopathy in the absence of coronary artery disease and hypertension. Various in vivo or in vitro measurements were performed during the study of these animal models. Type 1 diabetes (either streptozotocin-induced rats or genetic non-obese diabetic mice) (57-60) rodent models have been shown to have echocardiographic evidence of systolic and diastolic dysfunction (57,61), endothelial dysfunction (58), elevated LV end-diastolic pressure, reduced LV systolic pressure, cardiac output and cardiac power (62). However, studies on Type 2 diabetic, insulin resistant, obese Zucker rats have yielded conflicting results (63,64). It is likely that these

conflicting findings arose from different models used; as Type 1 and Type 2 diabetic rodent models have different cardiac energy metabolism and neurohormonal changes, resulting in different impact on cardiac function. The severity of cardiomyopathy may vary among different models depending upon the severity and duration of alterations of plasma parameters such as insulin level, leptin, glucose, fatty acids, cytokines, tumour necrosis factor-alpha (TNF-α). Therefore, experimental data obtained using animals models of diabetes should be used in caution when extrapolating to the human diabetes. Additionally, aetiology of cardiomyopathy, neurohormonal changes and severity of these alterations may vary between animal and patients with diabetes mellitus (65).

In a chronically pacing dog model, Nikolaidis et al have successfully shown us how CHF can lead to IR (66). Thirty-four conscious, chronically instrumented dogs were studied at four stages during the evolution of dilated cardiomyopathy (DCM) induced by rapid right ventricular pacing. They showed that severe DCM is associated with the development of myocardial and systemic IR. There was impaired myocardial glucose uptake and altered myocardial insulin signaling, involving decreased Ser 473 phosphorylation of Akt-1. Myocardial insulin resistance in advanced, severe DCM was also associated with reduced myocardial adenosine triphosphate (ATP) levels. There are no clear explanations of how IR leads to worsening of CHF. Various research groups have proposed a few possible hypotheses on the overlapping pathophysiological processes in both conditions over the last decade.

(1) SYMPATHETIC NERVOUS SYSTEM AND RENIN-ANGIOTENSIN ALDOSTERONE SYSTEM ACTIVATION

CHF invokes compensatory sympathetic nervous system (SNS) and renin-angiotensin aldosterone system activation, which leads to increase free fatty acids (FFA), thereby inhibits glucose uptake by muscle, and causes pancreatic damage. The increased plasma glucose elicits a compensatory insulin response but it is inadequate to compensate the hyperglycaemia because of the pancreatic damage mediated by cytokines such as TNF-α, angiotensin II and FFA. This leads to aberrant metabolism and IR. The increased FFA and glucose predispose to increased hepatic synthesis of triglycerides (TG) and increased angiotensin II, which in turn increases tissue TG levels and promote insulin receptor substrate-1 damage in the pancreas, thereby magnifies IR. Angiotensin II also promotes vasoconstriction, which in turn increase cardiac afterload (36). Angiotensin II activation and SNS activation are also common features of IR secondary to the compensatory hyperinsulinaemia (37,38).

(2) INFLAMMATION

Systemic abnormalities in CHF have become a focus of cardiology research lately. IR and CHF are increasing recognized as an inflammatory state. There is mounting evidence to suggest that these inflammatory mediators can predict the development of IR or diabetes in population at risk (39-42). Opie et al and Wisniaki et al (43) have both demonstrated that in insulin-resistant CHF patients, C-reactive protein, interleukin-6, TNF-α and its soluble receptors were all significantly elevated secondary to neuro-hormonal activation. High level of circulating TNF-α has been detected in cachectic patients with severe CHF

(44,45), and it has been shown to correlate with leptin level, disease severity and poorer exercise tolerance (46). At a molecular level, TNF-α increases the Ser phosphorylation of insulin receptor substrates results in reduction of auto-phosphorylation of tyrosine and tyrosine kinase activities. The docking and interaction of IRS to its downstream effectors and kinase such as PI3-K are markedly reduced, resulting in reduced glucose transport (47). These lead to IR and reduce myocytes' ability to utilize glucose as a substrate for ATP production. These maladaptive stress responses of IR and CHF have been recognized by Lip et al as a pro-thrombotic state, and can lead to thrombogenesis and poorer clinical outcome (48). They concluded in their study that IL-6 and tissue factors (but not vascular endothelial growth factor (VEGF), plasma viscosity, von-Willebrand factor, fibrinogen or soluble P-selectin) levels were predictors of mortality and poor prognosis in patients with CHF.

(3) ALTERED ADIPOKINES LEVELS

LEPTIN

Leptin, a product of ob-gene, has received a great deal of attention in recent research on metabolic syndrome and heart failure. Leptin is an adipose tissue specific protein with immune-modulatory properties. It plays an important role in weight regulations and energy expenditure. Leptin deficient mice have been shown to be susceptible to infections (49). Leptin release can be induced by pro-inflammatory cytokines and catecholamines (50,51). Besides IR/ diabetes (52,53) and CHF, elevated leptin levels were found in other

conditions with a high level of circulating proinflammatory cytokines and catecholamines such as obesity (54), sepsis (55,56) and chronic obstructive airway disease (57). Leptin stimulates fatty acid oxidation (58) and glucose uptake (59). It prevents lipid accumulation in non adipose tissue, which can otherwise lead to lipotoxicity (60). Lipotoxicity of pancreatic beta-cells, myocardium, and skeletal muscle leads, respectively, to type 2 diabetes, cardiomyopathy, and insulin resistance. High level of leptin has been consistently observed in patients with CHF (61,62) and correlates positively with IR (63) and levels of TNF-α (64). Doehner et al hypothesized that hyperleptinaemia may result in impaired cardiac energy metabolism. Serum level of leptin also correlates with the progressive functional impairment of advanced CHF (46). But interestingly leptin level was found to be low in patients with advanced CHF and cachexia (65,66). These findings may be the end results of excessive loss of muscle and fat mass in patients with advanced CHF.

ADIPONECTIN

Adiponectin, an adipocyte derived protein, which has anti-inflammatory, insulin sensitizing, anti-atherogenic properties (67). It also plays an important role in vascular remodeling. It has been researched extensively in various disease states lately particularly in patients with metabolic syndrome and CHF. Overweight individuals have reduced serum adiponectin levels. . Clinical studies have shown that adiponectin levels were low in patients with acute coronary syndrome (68) and T2DM (69), and were more closely related to the severity of insulin resistance and hyperinsulinaemia than to the degree of adiposity.

Paradoxically, adiponectin levels are higher in patients with CHF. It was not related to BMI in these patients (64). The levels of adiponectin also correlate positively with severity of CHF determined by NYHA functional class and serum NT-pro BNP levels. More importantly, elevated adiponectin levels were found to be a predictor of morbidity and mortality in CHF (70-72). As stated earlier, CHF is an inflammatory condition associated with elevated inflammatory markers. Of particular interest, serum adiponectin levels were negatively correlated with serum inflammatory markers such as highly sensitive C-reactive protein (hs-CRP) (73,74). Why are adiponectin levels high in CHF rather than low as in other disease states such as atherosclerosis and acute coronary syndrome? Owing to its anti-inflammatory and anti-diabetic properties, it has been speculated that the elevated adiponectin levels parallel the neurohormonal and inflammatory axes in the pathophysiology of CHF (71,75), as part of the physiological protective response to counteract inflammation and neurohormonal activation (71). Recent clinical studies have demonstrated the significance of adipocytokines modulation in HF patients (76). Van Berendoncks et al have shown that adiponectin mRNA expression was increased in CHF patients. Whereas its receptors (AdipoR1) and its downstream metabolic genes (i.e. PPAR–α and AMPK) expressions were decreased in CHF patients. More importantly, they have shown that four months of endurance resistance exercise training normalized these levels, suggesting that modulation of these adipocytokines altered glucose and lipids metabolism at the muscle level. This further consolidates our understanding of the beneficial effects of exercise training in CHF.

RESISTIN

We have now known that the adipose tissues play an essential part in the regulation of glucose and insulin metabolism through the release of adipocytokines. The adipocytokines also plays an important role in regulation of endothelial function and inflammation. Resistin has attracted a great deal of interest and attention in recent years. Resistin was originally described as an adipocyte-secreted peptide that induced insulin resistance in rodents (77). Administration of resistin to healthy rats impairs glucose tolerance and insulin action. Resistin has recently been shown to induce beta cell apoptosis in rats (78). There is increasing recognition of its role in the inflammation (79). Recent study has shown that resistin activates human endothelial cells through the up-regulation of cell adhesion molecules (80) and is a significant local and systemic regulatory cytokine involved in inflammation on vessels' walls in a rodent model (81). Serum resistin levels are increased in patients with T2DM. It is strongly associated with body mass index, and the degree of IR measured by HOMA-IR and various ED and inflammatory markers (82). More importantly, there is a strong positive correlation between resistin level and the development and degree of mircoangiopathies (i.e. retinopathy, neuropathy and nephropathy) in patients with T2DM independent of age, gender, BMI, and either the duration of T2DM ($P = 0.0318$) or serum creatinine ($P = 0.0092$) (83).

In a correlation study of the serum level of resistin and exercise capacity in patients with stable coronary artery disease, elevated serum resistin was also associated with poor exercise capacity and exercise-induced cardiac ischaemia. Adjustment for inflammatory markers attenuated these associations, suggesting

a possible role for resistin in inflammation and the pathophysiology of coronary heart disease (84). Long-term resistin over-expression was associated with a complex phenotype of oxidative stress, inflammation, fibrosis, apoptosis and myocardial remodeling and dysfunction in a rodent model (85). Therefore, with increased understanding of the pathophysiological role of resistin, it is not a surprise to see that serum resistin levels were associated with incident heart failure, even after accounting for prevalent coronary heart disease, obesity, and measures of insulin resistance and inflammation from the Framingham Offspring study (86). This study suggested the pathophysiological role of resistin in HF. Similar findings were shown from the Heart and Soul Study where patients with coronary heart disease with resistin levels in the highest quartile were at an increased risk of heart failure (hazard ratio [HR], 2.06; 95% confidence interval [CI], 1.26–3.39) and death (HR, 1.56; 95% CI, 1.11–2.18), adjusted for age, sex, and race. However, these effects were neutralised after adjusting for traditional cardiovascular risk factors such as obesity, hypertension, insulin resistance, dyslipidaemia, and renal dysfunction (87). Nonetheless, the current literatures seem to suggest that resistin plays an important role in vascular biology, inflammation and is highly related to the development of IR and the development of HF.

(4) FORMATION OF ADVANCED GLYCOSYLATION END PRODUCTS

This process is greatly accelerated in diabetic patients. High level of advanced glycosylation end products in the myocardium leads to increased collagen cross-linking and myocardial stiffness (88). This causes further deterioration of ventricular relaxation and contraction in patients with CHF.

Chronic metformin use has been shown to prevent the above process and improve ventricular function in canine diabetic models (89).

(5) HYPERINSULINAEMIA

Insulin is a catabolic hormone, and chronic hyperinsulinaemic state has been shown to increase myocardial mass and reduced cardiac output in rats (90). It can also lead to sodium retention (91) and subsequently decompensated heart failure. Besides that, hyperinsulinaemia also leads to activation of SNS (37), and increased pressor response to angiotensin II (38). Elevated catecholamine levels in CHF further antagonized insulin's actions, which promote lipolysis, results in increased free fatty acid and worsened insulin resistance (92) and cardiac energy metabolism. The results of these effects are increased cardiac hypertrophy, collagen formation, myocardial fibrosis (93), and eventually worsening of CHF.

(6) SUBSTRATE UTILIZATION

In a normal, unstressed heart, energy in the form of ATP is mainly derived from free fatty acids (FFA) oxidation (94). Under pathological stress, the heart will switch from FFA oxidation to more fuel-efficient glucose metabolism (95) (amount of ATP generated per molecule of oxygen consumed). IR is associated with a high level of circulating FFA. High supply of FFA exceeds the heart's oxidative capacity, leading to accumulation of intra-myocardial triglycerides, and hence lipotoxicity that worsens CHF (95-98). High level of

FFA impairs the heart's ability to utilize glucose as a main source of ATP generation in different ways:

1. It impairs insulin mediated glucose uptake through inhibition of insulin receptor substrates and protein kinase-B.
2. PPAR-α is activated, and this leads to the promotion of genes involved in FFA oxidation and pyruvate dehydrogenase kinase-4 (PDK-4), which inhibits pyruvate dehydrogenase (PDH) and influx of pyruvate into mitochondrial.
3. High level of acetyl-CoA from β-oxidation of FFA further activates PDK-4, leading to further inhibition of PDH, hence pyruvate influx.
4. Augmented acetyl-CoA also leads to accumulation of citrate, which subsequently inhibits phosphofructose kinase-1 (PK1), a rate-limiting enzyme of glycolysis.
5. Increased FFA level also correlates with decreased myocardial phosphocreatinine-to-ATP (PCr/ATP) ratios, suggesting impaired ATP production (99). ATP production depends on the energy of the proton gradient across the mitochondrial inner membrane. High level of FFA activates the transcription factors of PPAR, leading to increased expression of mitochondrial uncoupling proteins (UCPs) expression (100). UCPs are also being up regulated in heart failure (100). UCP lowers the proton gradient by allowing protons to re-enter the mitochondrial matrix with the production of heat rather than ATP (101).

In severe CHF, myocardial IR results in reduced membrane translocation of GLUT-4 (decreased glucose uptake) and decreased phosphorylation of Akt-1 (decreased glucose metabolism) resulting in decreased ATP production. It further prevents the heart adaptive response to stress (deriving ATP from glucose metabolism rather than FFA oxidation). CHF in the setting of IR is probably the worst of all possibilities for energy metabolism. Gene expression for metabolizing FFA is down regulated due to CHF, and genes for metabolizing glucose are down regulated secondary to IR, preventing the heart from utilizing either fuel (102,103).

(7) ENDOTHELIAL DYSFUNCTION (ED)

The endothelium represents an active and reactive single layer cells that line all the blood vessels in the body (104). A healthy endothelium should consist of a smooth surface that limits the activation of clotting cascades and pro-inflammation. Endothelial dysfunction is defined as inadequate or abnormal endothelial-mediated vasodilatation, which eventually leads to activation of coagulation and clotting cascades and inflammation, and eventually development of atherosclerosis. It was first observed in patients who underwent diagnostic coronary angiography (105). ED and IR often co-exist, and the combination of ED and IR were found among the individuals who are at higher risk of developing cardiovascular event (106). IR and ED represent the fundamental pathophysiological disturbance responsible for the clusters of metabolic and cardiovascular disease (107,108). The presence IR and ED are increasing recognised as a precursor to the development of atherosclerosis (109). ED leads to inadequate vasodilatation and/or paradoxical

vasoconstriction in coronary and peripheral arteries in response to stimuli that release nitric oxide (NO). Deficiency of endothelial-derived NO is believed to be the primary defect that links insulin resistance and endothelial dysfunction. NO deficiency can result from either decreased synthesis or accelerated degradation by high levels of reactive oxygen (ROS) and nitrogen (RNS) species, which are produced by cellular disturbances in glucose and lipid metabolism (110). ED is found in patients with insulin resistance prior to the development of diabetes, and is well described in obese patients, metabolic syndrome and in patients with gestational diabetes (111-113). ED was detected in patients with early asymptomatic HF (114) as well as symptomatic HF (115). The presence of IR was associated with ED in patients with CHF (116). Deficiency in endothelial-derived nitric oxide is believed to be the link between IR and ED (117). IR and ED have an impact on each other in terms of causation and outcome. There is a large body of evidence to show that IR is associated with ED (118,119). The exact mechanisms of how IR affects ED are not fully understood. Oxidative stress, hyperglycaemia, attenuation of insulin medicated NO release from vascular bed; dyslipidaemia and increased arginase activity have been suggested (120-122).

ED can impact on insulin action by altering its trans-capillary passage of insulin to target tissues, which results in abnormal tissues metabolisms, and accumulation of toxic metabolites, which in turns worsen IR. Vascular damage from oxidative stress and lipids deposition further induce localised inflammatory process that worsens IR and ED.

The presence of ED is also an important prognosticator in patients with established cardiovascular disease independent of other established cardiovascular risk factors (123). Therefore, improving ED and IR may represent therapeutic targets to prevent the development of atherosclerosis and cardiovascular disease.

CHAPTER SUMMARY

IR and CHF are an intricate pair of disease. There is increasing evidence to suggest that IR plays an important role in the pathophysiological processes in CHF. Activation of SNS and RAS, inflammation, altered adipocytokines levels, formation of advanced glycosylation products, changes of substrate utilization in the myocardium and endothelial dysfunction are possible explanations of how IR affecting disease process in CHF. With increased understanding of the pathophysiological role of IR in CHF, improving IR may potentially result in improvement of CHF.

CHAPTER 4: PHARMACOLOGICAL TREATMENT FOR INSULIN RESISTANCE AND CHRONIC HEART FAILURE

In this section, we will consider pharmacological tools that can be utilized to reverse IR in patients with CHF. We are mindful that lifestyle changes are important, most notably exercise which has been shown to improve IR in patients with impaired glucose tolerance in the Diabetes Prevention Program (DPP) study (124).

CHF DRUGS THAT IMPACT ON IR

A recent meta-analysis showed that drugs that inhibit the renin-angiotensin system might prevent the onset of diabetes mellitus (DM). In their meta-analysis, Andraws and Brown showed that in angiotensin converting enzyme (ACE) inhibitor trials, the odds of developing DM were reduced by 28% (OR 0.72, 95% CI 0.63 to 0.84, $p<0.001$), and in the angiotensin receptor blocker trials, there was a 27% reduction (OR 0.73, 95% CI 0.64 to 0.84, $p<0.001$) in the odds (125) compared to placebo or other antihypertensive agents such as calcium channel blockers and diuretics. With respect to beta-blockers, there may be differences between beta-blockers in their impact on insulin resistance (126). In the setting of CHF, carvedilol has shown superior effects in prevention of diabetes when compared with metoprolol in the COMET study (127).

Mineralocorticoid receptors are expressed in non-epithelial tissues, such as blood vessels, the heart and adipose tissue. Mineralocorticoid receptors

antagonist (MRA) had been shown to be beneficial in HF trials. There is increasing body of evidence to suggest the role of aldosterone in the pathophysiological process of HF, it is associated with fibrosis, worsening catecholamine process, inflammation and endothelial dysfunction (128). The Randomized Aldactone Evaluation Study (RALES) has shown mortality and morbidity benefit of adding spironolactone in addition to existing HF therapy during that time (129). Additionally, the newer MCA Eplerenone has also been shown to have striking mortality benefit in patients with LVSD following myocardial infarction (130) and also in milder HF population (131) in addition to RAS modulators (ACEi or ARB). Although both spironolactone and eplerenone are both MCAs, they have very different metabolic effects, particularly on IR or glycaemic control. In an animal model, the use of spironolactone was associated with an increased level of plasma aldosterone and impaired glucose tolerance compared to eplerenone (132). Whereas in a study examining the effects of eplerenone and spironolactone on cortisol and HbA1c levels in patients with chronic stable HF, plasma cortisol levels and HbA1c were significant higher, and adiponectin levels significantly decreased in patients taking spironolactone but remains unchanged in the eplerenone group (133). Eplerenone seems to have neutral metabolic effects on IR whereas spironolactone has been shown to upset glycaemic control in HF patients. Therefore, these metabolic effects should be considered when prescribing MCA to CHF patients with DM or IR.

DIABETIC DRUGS THAT IMPROVE IR

THIAZOLIDINEDIONE (TZDS)

TZDs (e.g. rosiglitazone and pioglitazone) belong to the high affinity ligands for the nuclear hormone receptor family member PPAR-γ (134). They modulate the transcription of the insulin-sensitive genes involved in the control of glucose and lipid metabolism in adipose, muscular tissue and in the liver. They improve insulin sensitivity in the liver and peripheral tissue, and reduce plasma insulin level and glycated haemoglobin. TZDs also improve lipid profiles and are associated with anti-inflammatory and anti-atherosclerotic properties (135). However, there have been reports of adverse cardiovascular outcomes associated with the use of TZDs, most notably with CHF hospitalizations. In a recent meta-analysis, Lago and colleagues reported that TZDs increased risk for development of CHF, probably as a result of fluid retention, across a wide background of cardiac risk (relative risk [RR] 1.72, 95% CI 1.21-2.42, p=0.002) (136). At this time, the Food and Drug Administration (FDA) indicates that TZDs are not recommended for use in patients with NYHA functional class III/IV CHF, and have introduced a 'black box' warning regarding the increased risk of CHF. There are additional concerns regarding the risk of myocardial infraction with TZDs especially with rosiglitazone. Recent results of different meta-analyses were inconclusive as to whether rosiglitazone caused real adverse effects of myocardial infarction (137,138). The US FDA have placed a 'black box' warning on rosiglitazone to signal potential of myocardial infarction and heart-related deaths until additional safety data is available. The UK Commission on Human

Medicines has reviewed the available data in year 2010 and has concluded that there is an increased cardiovascular risk for rosiglitazone. The Commission has therefore concluded that the benefits of rosiglitazone no longer outweigh its risks. The European Committee on Medicinal Products for Human Use has recommended the suspension of the marketing authorisations of rosiglitazone (Avandia, Avandamet) across the European Union.

METFORMIN

Another insulin sensitizing drug that may have potential use in CHF is metformin. Metformin has been on the market for almost 50 years and is widely prescribed in diabetic population. It was first described in the scientific literature in 1957 (139) and was first marketed in France in 1979, but did not receive approval by FDA for the treatment of Type 2 diabetes until 1994 in the USA . It is cheap and has potent insulin sensitizing effect (140). Metformin, according to the package insert, is contraindicated in all patients with ''heart failure requiring pharmacologic treatment'' because of increased risk of potentially lethal lactic acidosis (LA) (141). This stems from the previous reports of severe LA with phenformin, another biaguanide that was removed from the market after 306 cases of LA were reported in the 1970s. However, the available evidence of the risk of LA with metformin is somewhat lacking (142,143). Indeed, the reported incidence of LA related to metformin have been extremely low in large observational studies and metformin levels do not correlate with lactate levels in individuals who develop LA which may support the notion that metformin may be 'an innocent bystander'' in sick patients

rather than the causal agent (144,145). The overall rate of LA with metformin has been estimated at 6.3 cases per 100,000 patient-years (143).

With respect to studies reporting the use of metformin in CHF, there has been several analyses of prescribing databases. In a retrospective cohort analysis by Masoudi et al (28), 16417 Medicare beneficiaries with diabetes discharged after hospitalization with the principle discharge diagnosis of heart failure were assessed. The primary outcome of the study was time to death due to all causes. Secondary outcomes included time to readmission for all causes or for heart failure. Crude 1-year mortality rates were lower among the 2226 patients treated with TZDs (30.1%) or the 1861 treated with metformin (24.7%) compared with that among the 12069 treated with neither insulin-sensitizing drug (36%, P=<0.0001 for both comparisons)(Figure 10). The study concluded that these treatments are not associated with increased mortality and may improve outcomes in older patients with diabetes and heart failure. Admissions for all causes did not differ with either insulin sensitizer. There was a higher risk of readmission for heart failure with TZDs treatment (HR 1.06, 95% CI 1.00 to 1.09) and lower risk with metformin treatment (HR 0.92, 95% CI 0.92-0.99). Notably, there was no excess of admissions for LA in patients treated with metformin.

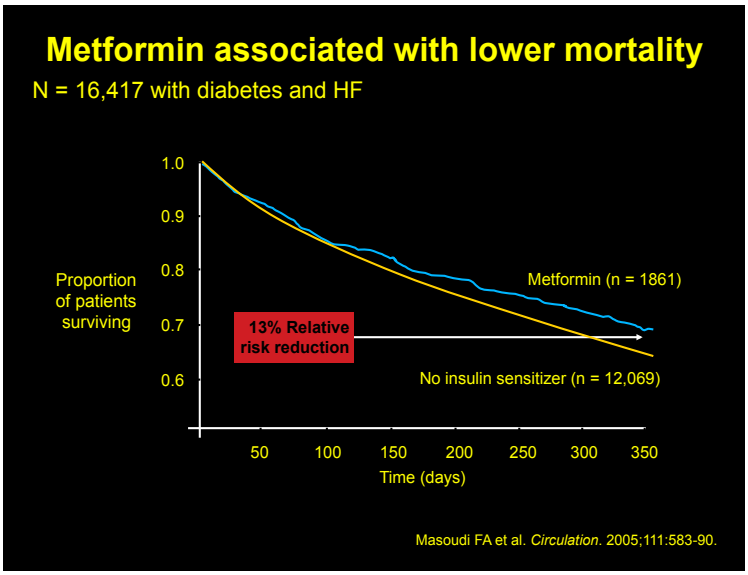

Figure 10: Observation data suggests that metformin may have mortality benefit in Type 2 Diabetes Mellitus. Masoudi et al. Circulation 2005;111:583-90

Eurich DT and colleagues had analyzed the Saskatchewan Health database to identify patients with CHF and diabetes for a retrospective analysis (146). 12272 new users of oral anti-diabetic agents were identified. The average follow-up was 2.5 years. Compared with sulfonylurea monotherapy, fewer deaths occurred in subjects receiving metformin: 404 (52%) for sulfonylurea monotherapy versus 69 (33%) for metformin monotherapy (HR 0.70 [95% CI 0.54-0.91]) and 263 (31%) for combination therapy (0.61 [CI 0.52-0.72]). Reductions in deaths or hospitalization were also noted in metformin treated groups. Therefore, metformin, alone or in combination, in subjects with heart failure and Type 2 diabetes was associated with lower morbidity and mortality compared with sulfonylurea monotherapy.

GLUCAGON LIKE PEPTIDE (GLP-1) AND DIPEPTIDYL-PEPTIDASE-4 INHIBITORS

The incretin system has received a great deal of attention in the treatment of diabetes mellitus. Glucagon like peptide (GLP-1) is a gut derived incretin hormone that has glucose-dependent insulinotropic effects. GLP-1 stimulates insulin release, suppresses glucagon secretion, inhibits gastric emptying and reduces appetite and food intake. The current approach to enhance incretin system is either to increase the incretin level (incretin mimetic, i.e. GLP-1) or to reduce the degradation of incretin (dipeptidyl-peptidase-4 inhibitors (DPP-4), i.e. sitagliptin and vildagliptin). The incretin concept was developed from an observational study in the 1960's, when enteral nutrition was shown to be a more potent insulinotropic stimulus than iso-glycaemic intravenous challenge (147). GLP-1 levels increase rapidly after eating but also inactivated swiftly by DPP-4. Activation of incretin systems lead to insulin biosynthesis, stimulation of β- cell proliferation (148), promote resistance to apoptosis and enhanced β-cell survival (149,150). Nikolaidis et al shared some insights into how GLP-1 affects cardiac function from canine models. He demonstrated that recombinant GLP-1 infusion resulted in dramatic improvement of LV and systemic haemodynamics in conscious dogs with advanced dilated cardiomyopathy induced by rapid pacing (151). This was followed by a clinical study, which showed improvement of LV ejection fraction, functional status, and reduced brain natriuretic peptide (BNP) in patients with CHF (95). The expected insulinotropic effects of GLP-1 were observed in this study. Plasma glucose and non-esterified fatty acid were significantly reduced in GLP-1 group, in association with an increase in plasma insulin levels but

measurement of insulin resistance (insulin-mediated glucose uptake) were not performed in this study. GLP-1 and DDP-4 inhibitors have not been shown to ameliorate IR directly to date. Therefore, improvement of cardiac function may not be a direct effect of GLP-1 on IR. It may be the results of improvement of endothelial function (152,153), GLP-1 variable inotropic effects (154,155) and more efficient myocardial ATP production from reduced myocardial FFA.

It should be noted that the available DPP-IV inhibitors, which are currently used in the treatment of diabetes mellitus, could increase GLP-1 levels (156). To date, it has not been shown to have any cardioprotective effects post myocardial infarction (157) although smaller animals studies have show positive effects on BNP expression (158) and improvement of cardio-renal function in a porcine models (159).

A few randomized controlled trials are currently underway to define the utility of targeting the incretin system in HF patients with DM. Incretin-based therapy may represent a novel therapeutic strategy in the treatment of HF patients with diabetes, in particular for their cardioprotective effects independent of those attributable to tight glycaemic control.

CHAPTER SUMMARY

Lifestyles changes such as diet and exercise are important in improving IR in CHF patients but difficult to achieve and prescribe. CHF trials seem to suggest that most conventional CHF medications have favourable impact on glycaemic control or prevent the development of diabetes. In terms of diabetic medications, insulin sensitizers are limited to TZDs and metformin. The use of TZDs have reduced greatly because of risk of exacerbating HF and its association with increased risk of myocardial infarction, whereas metformin is increasing recognised as a safe and beneficial drug in T2DM and CHF from large observational studies. Lastly, our increased insights and understanding of the incretins system has opened a new horizon in the potential treatment options in CHF, and outcome trials are awaiting.

CHAPTER 5: METFORMIN USE AND MORTALITY IN PATIENTS WITH CHF

INTRODUCTION

Chronic heart failure (CHF) and type 2 diabetes mellitus (T2DM) frequently coexist (160-162). In population based studies and in CHF trials, the prevalence of T2DM in patients with CHF is estimated to be between 11% and 28% and among all patients hospitalized for CHF it has been reported that 25-30% have T2DM. This combination can be lethal since diabetes has consistently been shown to be an independent predictor of increased morbidity and mortality in patients with either symptomatic heart failure or asymptomatic left ventricular dysfunction (163,164).

Unfortunately, patients with a combination of CHF and T2DM have limited drug therapy options for their diabetes. Because insulin resistance is a key pathophysiological process in these patients (165), it would seem intuitive to administer an insulin-sensitizing agent such as a thiazolidinedione or metformin. However, thiazolidinediones have the potential to exacerbate CHF in patients with poor cardiac reserve and are currently contraindicated in CHF patients with New York Heart Association (NYHA) functional class III or IV. While metformin is a widely prescribed drug in the management of T2DM, its use in patients with T2DM and CHF has previously been discouraged due to concerns over the risk of lactic acidosis originating from earlier experience with phenformin. This was withdrawn from U.S. market in the 1970s due to case

reports of fatal lactic acidosis. However, both clinical experience and the literature suggest that the risk of metformin–associated lactic acidosis is very low, and similar to that of other anti-diabetic drugs (28,166). In fact, observational data suggest that metformin may actually be beneficial for CHF through its insulin sensitizing properties. A retrospective cohort study in a group of Medicare recipients discharged from U.S. hospitals with a principal diagnosis of CHF, suggests that metformin (and thiazolidinediones) were not associated with increased mortality and may improve outcomes in older patients with T2DM and CHF (28). It has been proposed by several groups that compensated CHF can no longer be upheld as contraindication for metformin (167-169). The beneficial effect of metformin in CHF may extend to patients with other manifestations of cardiovascular disease; an observational study in the UK demonstrated that cardiovascular risk was lower among metformin users compared to sulphonylureas users (169). Further evidence for the potential benefit of metformin in patients with both T2DM and CHF may result in expanding the therapeutic options for managing this important common and complex group of patients. Therefore, the aim of this study was to add to the growing body of evidence on the safety of metformin, and to explore benefits of its use among patients with T2DM and CHF in Tayside, Scotland.

AIMS AND OBJECTIVES

To investigate the use of metformin therapy for treating type 2 diabetes mellitus (T2DM) with Chronic Heart Failure (CHF) in a large population-based cohort study.

METHODS

DATA SOURCES

This study was carried out in the population (approximately 400,000) of Tayside in Scotland, using the Diabetes Audit and Research in Tayside Scotland (DARTS) (171) information system and the dispensed prescribing database maintained by the Health Informatics Centre (HIC) of the University of Dundee (formerly known as the MEMO database). This contains records of all dispensed community prescriptions dating back to 1993. The DARTS dataset contains detailed clinical information on every patient diagnosed with diabetes in Tayside. Clinical information is collected according to the national clinical dataset for the care of diabetic people in Scotland, and includes diabetes type, date of diagnosis, duration, therapy, HbA_{1c}, presence (and date) of microvascular and macrovascular diabetic complications and cardiovascular risk factors.

Datasets available from HIC also include hospital discharge data, biochemistry data, mortality data, socio-demographic descriptors, and other data that are linked by a unique 10 digit patient identifier, the community health index (CHI) number that is used for all health care activities in Tayside. The first six digits are the date of birth, with a figure coding sex (odd male, even female) in the remaining four distinguishing digits. This number facilitates high accuracy record linkage at the level of the individual across the region. All research data are robustly anonymised and approved by the Tayside NHS

Caldicott Guardians. The study was granted ethical approved by the Tayside Committee on Medical Research Ethics.

STUDY POPULATION

The study population was defined as residents of Tayside who were registered with their GP during the study period 1994-2003. Using the DARTS database, we identified all Tayside residents who were diagnosed with T2DM prior to December 2003. We then identified all those who were newly treated with oral hypoglycaemic agents during the study period (January 1994 to December 2003). Any patients who received oral hypoglycaemic agent prescriptions before 1994 or received insulin at any point during the study period were excluded.

We identified patients who had incident CHF during the study period and defined a date of CHF diagnosis for each patient. This was the earliest date of patients fulfilling any one of the following criteria: 1. Patients with a hospital admission ICD9/10 diagnostic code for CHF during the study period (ICD-9 428 ICD-10 50). The date of admission was defined as the date of CHF diagnosis. 2. Patients commenced on CHF medications defined as a combination of loop diuretics and ACEi. Co-prescribing had to occur within a 90-day period, and the date of the second drug was defined as the date of CHF diagnosis. 3. Patients who had at least one admission for myocardial infarction and then received loop diuretic medication. The date of diuretic medication was defined as the date of CHF diagnosis. Patients were excluded if their plasma creatinine concentration was > 200 μmol/L prior to the prescribing of a loop diuretic. This

was to exclude patients with renal hypertension without CHF who might receive loop diuretics and ACE inhibitors. We have previously used similar criteria to identify CHF from large datasets in Tayside (172).

To be eligible for the study, the date of CHF diagnosis had to occur after the date of diagnosis of T2DM. The patient also had to receive a first prescription for either metformin or sulphonylureas within 1 year after their date of CHF diagnosis. Patients were then divided into three cohorts: those who received prescriptions for metformin only (metformin monotherapy), those who received prescriptions for sulphonylureas only (sulphonylureas monotherapy) and those who received prescriptions for both (combination).

STATISTICAL ANALYSIS

All subjects were prospectively followed up from their index date (the date of CHF diagnosis) until the primary outcome, termination of HIC health care coverage, or termination of the study. The study outcomes were all-cause mortality, both at 1 year (short term), and by the end of the follow-up period (long term). All cause mortality was determined from death certification records of the General Register Office (GRO) of Scotland. We compared survival between cohorts for each outcome using Kaplan-Meier survival plots and used Cox regression analyses to estimate the relative risks of each outcome for patients in the study cohorts. For this analysis, the metformin monotherapy cohort and the combination cohort were merged into a larger cohort, with the sulphonylureas monotherapy cohort as the reference group. Survival times were censored if patients left Tayside, or at the end of the study period. The

following confounding variables were investigated: sex, age at index date, duration of diabetes at index date, creatinine (divided into quartiles in ascending orders) and HbA1c. We also accounted for whether the patient had been admitted to hospital with an ICD9/10 diagnostic code for a major cardiovascular event (myocardial infarction, coronary heart disease or stroke) prior to their diagnosis with CHF. Finally, we calculated the proportions of patients in each cohort who had received prescriptions for any of four drug types: angiotensin-converting enzyme inhibitors aspirin, diuretics or beta-adrenoceptor blocking drugs (beta blockers). Continuous covariates were categorised into quartile groups where appropriate, and all covariates were included in the final models only if they were statistically significant in univariate analyses ($p < 0.05$), to produce adjusted risk estimates for all covariates. All analyses were conducted using SPSS version 16.0 (Chicago, USA).

RESULTS

There were 1,141 patients who were diagnosed with T2DM prior to December 2003, who received oral hypoglycaemic agents during the study period (1994 to 2003) but were not on insulin. All of these patients had been admitted to hospital with a diagnostic code for CHF, although 218 patients were not eligible for the study as their hospital admissions occurred out with the study period. It was not possible to identify a date of diagnosis of CHF for 9 patients who were also excluded from the study. From the remaining 914 patients, we identified 769 whose date of CHF diagnosis occurred after date of diagnosis of T2DM.

Four hundred and ninety patients were prescribed either metformin or sulphonylureas after their date of CHF diagnosis. However, we excluded a further 59 patients whose first oral hypoglycaemic agent was prescribed more than 365 days after date of CHF diagnosis, and 9 without a valid date of diagnosis of T2DM. Of the remaining 422 patients (mean age 75.4 ± 0.56yrs), 68 were prescribed metformin only, 217 were prescribed sulphonylureas only and 137 received prescriptions for both. The characteristics of these patients are presented in Table 5. We compared patients who had received any metformin (metformin monotherapy cohort or combination cohort) with patients in the sulphonylureas monotherapy cohort. Although they were slightly younger and more likely to be female, the only statistically significant differences were that they had lower mean creatinine and a higher proportion was treated with aspirin and ACE inhibitors.

Both 1-year and long-term mortality were higher in the sulphonylureas monotherapy cohort compared with patients prescribed metformin. In the Cox regression analysis, the unadjusted hazard ratios were 0.56 (95% CI 0.38-0.84) and 0.53 (95% CI 0.33-0.67) for 1 year and long-term mortality respectively, when the metformin and combination cohorts were grouped together and compared with the sulphonylurea monotherapy cohort (Table 6). After adjusting for baseline differences between the two groups, users of metformin, alone or in combination had a 30-40% lower risk of the outcomes (Table 6 and Figure 11).

DISCUSSION

Metformin is a widely prescribed potent insulin-sensitizing drug, which is cheap, and has been on the market for almost 50 years. However, in the relatively large group of T2DM patients with concomitant CHF, it is largely contraindicated. According to the package insert, metformin is contraindicated in patients with CHF requiring pharmacological therapy due to a possible increased risk of lactic acidosis. This is due to historical experience of lactic acidosis with phenformin, despite the fact that metformin dose not predispose to this when compared with other therapies (167). Other contraindications such as old age, renal impairment and CHF are increasingly disregarded in clinical practice. The key finding of this study is that patients with T2DM and CHF who were treated with metformin alone or in combination with sulphonylureas were at significantly lower risk of all cause mortality during 1 year and long-term follow-up than those who were treated with sulfonylurea alone. This remained so even after full correction for multiple possible confounding influences. This reduces the possibility that this finding might be due to differences in baseline co-morbidities, medication or other patient characteristics that may influence channelling bias.

How does metformin mediate these beneficial cardiovascular effects? Metformin has potent insulin sensitizing effects although its precise mechanisms of action are not fully understood (140). Arguably, the insulin sensitizing properties might confer in part some of the beneficial effects. CHF is increasingly recognized as an insulin resistant state (165,173). Studies that

have demonstrated an association between CHF and insulin resistance have found that the degree of insulin resistance is independently associated with the severity and exercise intolerance in CHF in terms of exercise capacity and peak oxygen consumption (VO_2) or the 6-min walk test (116,174,175). Insulin resistance also predicted mortality in patients with CHF, independent of body composition and other established prognostic indicators (35). These findings support the notion that insulin resistance is pathophysiologically linked with CHF and is implicated in the disease progression. This is likely because insulin resistance is associated with endothelial dysfunction, inflammation, increased oxidative stress and myocardial remodelling; processes that accelerate the progression of disease in CHF. Therefore, it is likely that the observed beneficial effects of metformin might be related to its potent insulin sensitizing effects (176,177). Indeed, metformin has been shown to offer protection from cardiovascular disease in general (140,177,178). In the UK Prospective Diabetes Study (UKPDS), metformin decreased the risk of mortality and morbidity among obese patients with type 2 diabetes who had cardiac disease (179). In keeping with our finding that CHF patients in the metformin monotherapy group or in combination had better survival compared to those on sulfonylurea alone, evidence from previous studies showed clearly that its benefits outweigh its risks in patients with haemodynamically stable heart failure and adequate renal function (28,166).

It should also be noted that metformin activates 5'-AMP-activated protein kinase (AMPK), a heterotrimeric enzyme that is expressed in many tissues, including the heart and vasculature (137). In animal models, cardiac

AMPK has been shown to mediate ischaemic glucose uptake and prevent post-ischaemic cardiac dysfunction (180). Metformin may also have specific vasculo-protective effects and can improve endothelial function (181).

Our study is observational and in common with all studies of this nature, it is impossible to account for all possible confounding influences that may have biased the observed differences between the groups considered. For example, patients in the sulphonylurea monotherapy group were somewhat older and have more prevalent use of loop diuretics. This may imply that this group have greater prevalence of left ventricular dysfunction and degree of heart failure than the metformin cohort. This may well have led to non-prescription of metformin given the well-known perception of contraindication of this agent in patients with heart failure. In this context, the presence of left ventricular dysfunction could easily lead to non-prescription of metformin, and therefore, confounding by contraindication. Patients in the sulphonylurea group have higher creatinine level, which is another potential confounder. Metformin is contraindicated in patients with renal impairment and physicians would be likely to withhold metformin from patients with the worse renal function, a well recognised independent predictor of adverse outcome in patients with heart failure. Lastly, the findings of lower hazard ratios in patients with higher creatinine is counterintuitive, this may be a reflection of higher usage of diuretics and ACE inhibitors. However, this analysis is based on one creatinine measurement and therefore, evidence of higher creatinine level associated with better outcome in heart failure patients with T2DM must remain speculative and cannot be inferred directly from this study. Clearly, further randomised

placebo controlled trials in this area would be required to provide definitive evidence of the benefit of metformin in this group of patients and further define the underlying mechanisms.

CONCLUSION

This large observational data suggest that the insulin sensitizer metformin is probably more effective than sulphonylurea based monotherapy in the treatment of CHF in patients with T2DM. A clinical trial of metformin in CHF is warranted to corroborate the observational study results

CONTRIBUTIONS TO STUDY

This study was published in the American Journal of Cardiology in year 2010. PMID: 20854965. The first author of the paper is Dr JM Evans. I contributed to the conception and design of the study, analysis of data and interpretation, manuscript writing and revision

ACKNOWLEDGEMENTS

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Table 5: Characteristics of patients in the study cohorts with p values for differences between the 'any metformin' cohort and the sulphonylureas monotherapy cohort.

	Sulphonylureas monotherapy	Metformin monotherapy	Metformin + Sulphonylurea Combination	Any metformin: Metformin monotherapy AND Combination	p*
Total	217	68	137	205	
% female	45.2	48.5	46.7	47.3	0.66[1]
Age in yrs (SD)	76.7 (SD 9.8)	75.5 (SD 8.9)	73.4 (SD 8.7)	74.1 (SD 8.8)	0.19[2]
Mean diabetes duration in yrs. (SD)	6.7 (SD 6.2)	6.7 (SD 6.2)	7.7 (SD 5.8)	7.4 (SD 5.9)	0.86[2]
Previous hospital admission (%)	72 (33.2%)	23 (33.8%)	37 (27.0%)	60 (29.3%)	0.39[1]
Mean last creatinine µmol/L (SD)	170.8 (SD 139.4)	135.1 (SD 71.0)	133.2 (SD 82.8)	133.8 (SD 78.0)	<0.001[2]
Mean last HbA1c (SD)	7.20 (SD 1.6)	7.35 (SD 1.5)	7.59 (SD 1.9)	7.51 (SD 1.8)	0.56[2]
% on ACE inhibitor	32.6	41.8	43.3	42.9	0.03[1]
% on aspirin	51.6	68.7	67.6	68.0	0.001[1]
% on diuretic	**21.9**	**14.9**	**17.6**	**16.7**	**0.13[1]**
% on beta-blocker	11.6	19.4	15.4	16.7	0.13[1]

Pearson Chi square[1]. Independent samples t test[2]

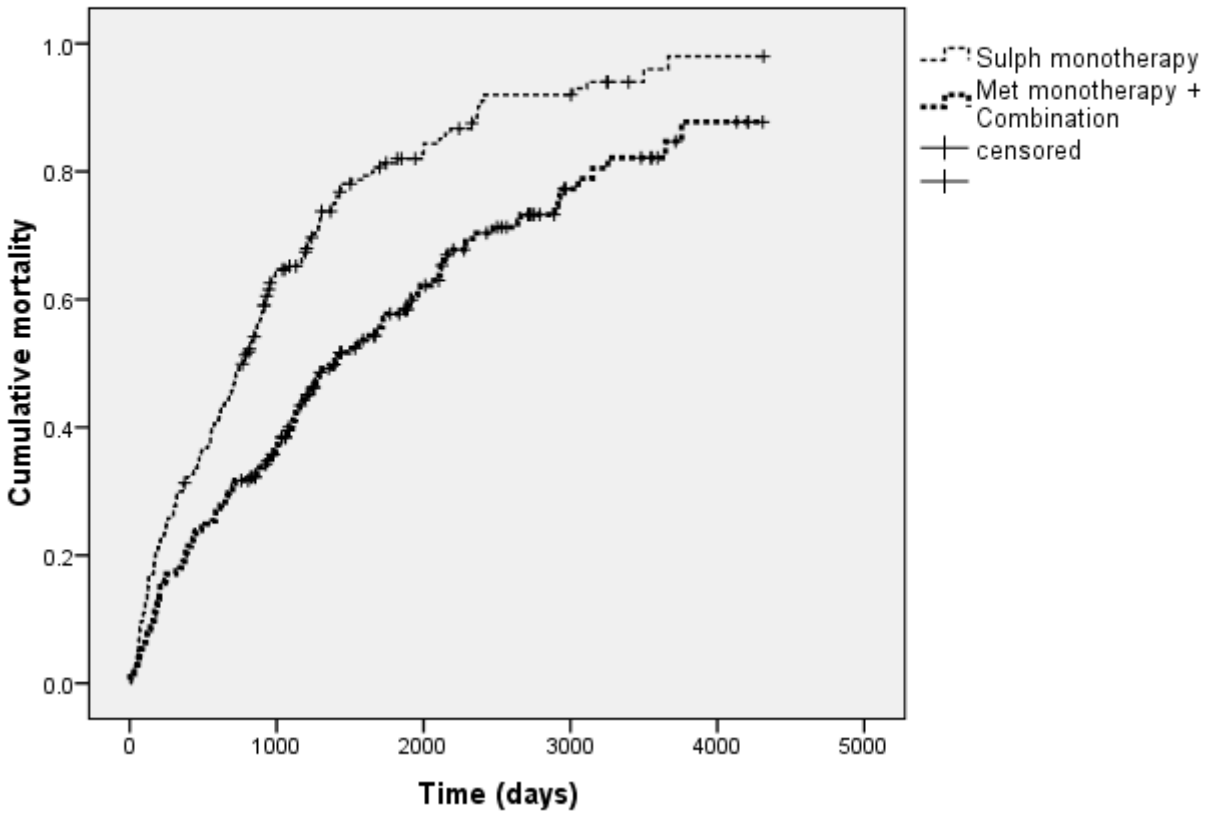
* p comparison is between sulphonylurea monotherapy vs 'Any metformin: Metformin monotherapy and Combination'

Table 6: Cox regression analysis showing unadjusted and adjusted odds ratios (with 95% confidence intervals) for all covariates for 1- year and long-term mortality.

	1- Year mortality		**Long-term mortality**	
Cohort	Unadjusted	Adjusted[1]	Unadjusted	Adjusted[1]
Sulphonylurea monotherapy	1.00	1.00	1.00	1.00
Metformin monotherapy + Combination	0.56 (0.38-0.84)	0.60 (0.37-0.97)	0.53 (0.33-0.67)	0.67 (0.51-0.88)
Sex				
Male	1.00	1.00	1.00	1.00
Female	0.82 (0.67-1.00)	0.47 (0.29-0.77)	1.16 (0.93-1.45)	0.64 (0.49-0.84)
Age				
< 60 yrs.	1.00	1.00	1.00	1.00
60-69 yrs.	5.60 (0.76-41.4)	4.62 (0.62-34.64)	1.15 (0.57-2.32)	1.11 (0.52-2.39)
70-79 yrs.	5.07 (0.70-36.9)	4.20 (0.59-34.64)	1.66 (0.85-3.28)	1.57 (0.75-3.25)
80-89 yrs.	7.06 (0.97-51.5)	7.33 (0.98-54.86)	2.82 (1.43-5.56)	2.61 (1.24-5.49)
>89 yrs.	8.57 (1.09-67.7)	12.39 (1.52-101.06)	3.24 (1.51-6.94)	3.44 (1.47-8.05)
Duration				
< 5 yrs.	1.00	-	1.00	1.00
5-9 yrs.	1.44 (0.91-2.28)	-	1.26 (0.97-1.64)	0.92 (0.67-1.26)
10-14 yrs.	1.70 (1.00-2.91)	-	1.28 (0.92-1.78)	1.13 (0.77-1.64)
>14 yrs.	1.38 (0.74-2.58)	-	1.80 (1.25-2.58)	1.77 (1.17–2.65)
Previous hospital admission				
Yes v No	1.23 (0.83-1.83)	1.08 (0.67-1.74)	1.01 (0.79-1.28)	0.97 (0.73-1.27)
Creatinine[1]				
Quartile 1	1.00	1.00	1.00	1.00
Quartile 2	0.54 (0.30-0.96)	0.46 (0.26-0.84)	0.55 (0.38-0.80)	0.52 (0.35-0.76)
Quartile 3	0.29 (0.14-0.59)	0.23 (0.11-0.47)	0.62 (0.43-0.88)	0.53 (0.36-0.77)
Quartile 4	0.67 (0.38-1.17)	0.51 (0.28-0.91)	1.09 (0.78-1.53)	0.87 (0.61-1.23)

HbA1c[2]				
< 7.5	1.00	-	1.00	-
7.5 – 9.49	1.14 (0.70-1.83)	-	1.08 (0.83-1.40)	-
>9.49	1.06 (0.48-2.34)	-	1.03 (0.67-1.57)	-
ACE				
Yes v No	0.56 (0.36-0.86)	0.57 (0.34-0.97)	0.66 (0.52-0.84)	0.76 (0.58-1.00)
Aspirin				
Yes v No	0.61 (0.41-0.89)	0.67 (0.42-1.08)	0.65 (0.52-0.82)	0.80 (0.61-1.05)
Diuretic				
Yes v No	1.14 (0.72-1.81)	-	1.09 (0.83-1.43)	-
Betablocker				
Yes v No	0.46 (0.22-0.94)	0.41 (0.16-1.04)	0.52 (0.36-0.74)	0.52 (0.34-0.80)
Available for 346[1] and 363[2] patients				

Figure 11: Kaplan-Meier plot for 1-year follow-up, comparing mortality in the sulphonylureas cohort with mortality in the any metformin cohort.



CHAPTER SUMMARY

Insulin resistance and CHF is an intricate pair that forms a vicious cycle that worsens each other at both tissue and cellular levels. Our increased understanding of the relationships between IR and CHF provides the rationale for targeting IR in the development of new CHF therapy. However, there appear to be some conundrum in the choice of available insulin sensitizers as there are safety issues of regarding TZDs and metformin. While the concerns regarding TZDs appear to be justified, there is now emerging evidence that metformin may not only be safe in CHF but may indeed be good in CHF. However, prospective trials are needed to prove this. TAYSIDE study (MeTformin in Insulin ResistAnt LV DYSfunctIon, Double blind, placEbo-controlled trial) will determine if reversing IR with metformin will have beneficial effects in patients with CHF.

CHAPTER 6: THE EFFECTS OF METFORMIN ON INSULIN RESISTANCE AND EXERCISE PARAMETERS IN PATIENTS WITH HEART FAILURE

INTRODUCTION

Chronic heart failure (CHF) is an insulin resistant (IR) state (165). We and others have shown that IR is highly prevalent among non-diabetic patients with CHF (116,174,182), and the degree of IR correlates with disease severity and outcome (35,116). Furthermore, it is associated with reduced exercise capacity (116,175). It is unclear whether IR is a bystander reflecting disease severity or whether it is a culprit contributing to the pathophysiology of CHF. Previous association studies did not distinguish the cause and effect. Therefore, a proof of concept study is required to test the hypothesis that IR is a culprit, and that reversing IR will lead to clinical improvement in CHF. However, the numbers of insulin sensitizers that are suitable to be tested are limited. Thiazolidinediones have the potential to exacerbate CHF in patients with reduced cardiac reserve. Although metformin is a widely prescribed drug in the management of T2DM, and its use in diabetic patients with CHF has previously been discouraged due to concerns over the risk of lactic acidosis originating from earlier experience with phenformin. However, clinical experience suggests that the risk of metformin–associated lactic acidosis is very low and similar to that of other anti-diabetic drugs (183). Indeed, there is observational data to suggest that metformin may actually be beneficial for CHF (18,184). We

have therefore conducted a proof of concept study to evaluate the impact of metformin on IR and its effects on exercise capacity in non-diabetic patients with CHF.

RESEARCH DESIGN AND METHODS

This is a randomized, placebo-controlled trial designed to evaluate the impact of metformin on IR and its effects on exercise capacity in non-diabetic patients with CHF. The primary endpoint of the trial was to determine if improvement of IR with metformin lead to improvement of peak VO_2. However, many patients with CHF are unable to perform maximal exercise, and oxygen requirements for activities of daily living rarely approach maximal levels (185). Therefore, we have included the sub-maximal derived exercise variable of the slope of the ratio of minute ventilation to carbon dioxide production (VE/VCO_2) as a secondary end-point of this study. VE/VCO_2 slope is an index of ventilatory response to exercise, unlike peak VO_2, VE/VCO_2 is not influenced by the mechanical work done during exercise testing but reflects alterations in the peripheries caused by the disease in CHF, which can in turn lead to the progression and symptomatology of CHF (186). Furthermore, we have also explored possible mechanisms of improvement of exercise capacity by measuring left ventricular ejection fraction by echocardiography, endothelial function and related biomarkers. The study was approved by the East of Scotland Research Ethics Service (07/S1401/59).

PATIENT POPULATION

Every patient provided written informed consent prior to participation in this study. Patients with CHF were recruited from out patient cardiology clinics and local echocardiography database. Patients with a history of CHF and left ventricular systolic dysfunction on echocardiography had enrolled for a fasting blood test to determine IR status by an empirical fasting insulin resistance index (FIRI), consisting of the product of plasma insulin and glucose divided by 25. CHF patients with a FIRI $\geq$2.7 were considered to have IR (116) and invited to participate in the study. Exclusion criteria included: patients with history of type 2 diabetes or fasting glucose of more than 7mmol/L; patients aged >80 years; patients with NYHA functional class IV and decompensated CHF; renal dysfunction (serum creatinine > 160 μmol/L); patients unable to exercise including patients that were excluded for reasons of safety or potential effects on exercise performance. Patients had to be on stable dose of CHF medications at least one month prior to screening. 127 patients were screened (Figure 12) and 53 patients were excluded based on exclusion criteria, and 21 were found to be non-IR. 62 patients with evidence of CHF and IR were randomised to receive metformin or placebo (2:1 ratio) with 39 patients randomised to receive metformin and 23 to receive a placebo. Treatment allocation was masked for patients and investigators until after database lock. Compliance was assessed by tablet counting.

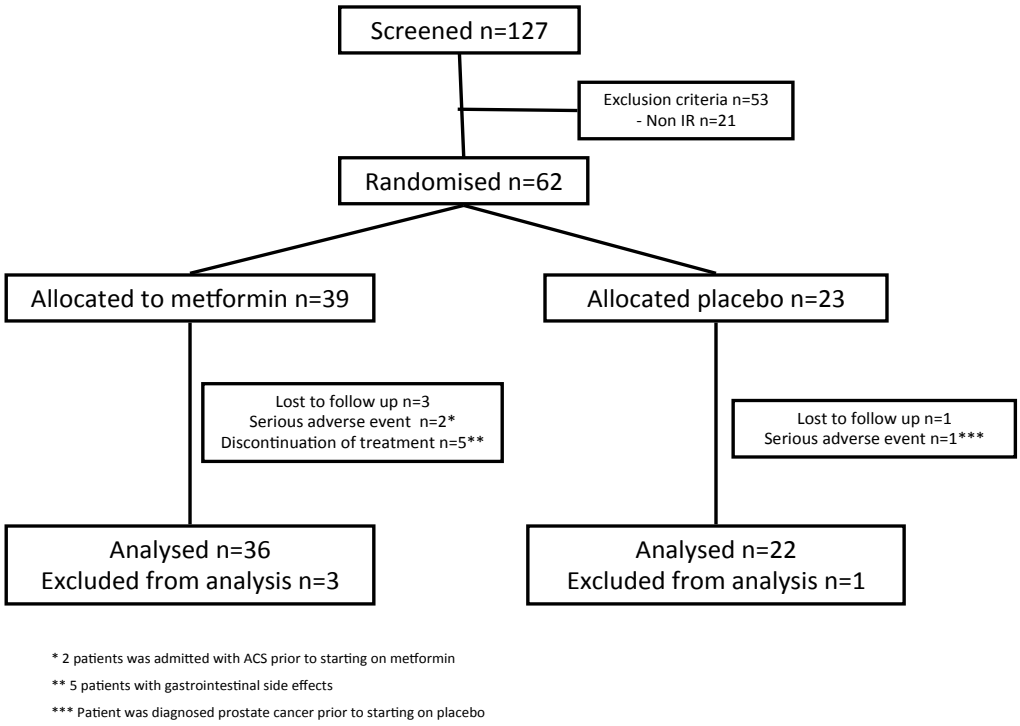


Figure 12: The CONSORT (Consolidated Standards of Reporting Trial) diagram describing outcomes of all patients within the study

STUDY PROTOCOL

The study consisted of 6 visits (Figure 13). At visit 1, patients underwent physical examination, fasting blood tests, cardiopulmonary exercise testing (CPET), two-dimensional echocardiography (2-D echo), endothelial function assessments, 6-minute un-encouraged walk test (6MWT) and Minnesota Living with Heart Failure Questionnaires (MLHF). CPET was repeated at a separate visit (Visit 2) within a week in order to achieve consistent exercise parameters with a variation of exercise duration of less than 15% prior to randomization. Following this visit, patients were randomized to receive either 4 months of metformin (1000mg b.d.) or matching placebo using a pre-established computer generated sequence from our study drug provider (Western

Infirmary, Glasgow). The dose and duration of metformin therapy was based on a previous study in patients with impaired glucose tolerance, which showed that this dose regimen was well tolerated and had a beneficial effect on endothelial function (187). The metformin study drug was commenced at 500mg b.d. for two weeks and was up titrated if well tolerated based on symptoms and measurement of plasma lactate and renal function. At subsequent visits, patients were reassessed and doses of study drug altered according to tolerability. All measurements of interests were repeated after 4 months of intervention.

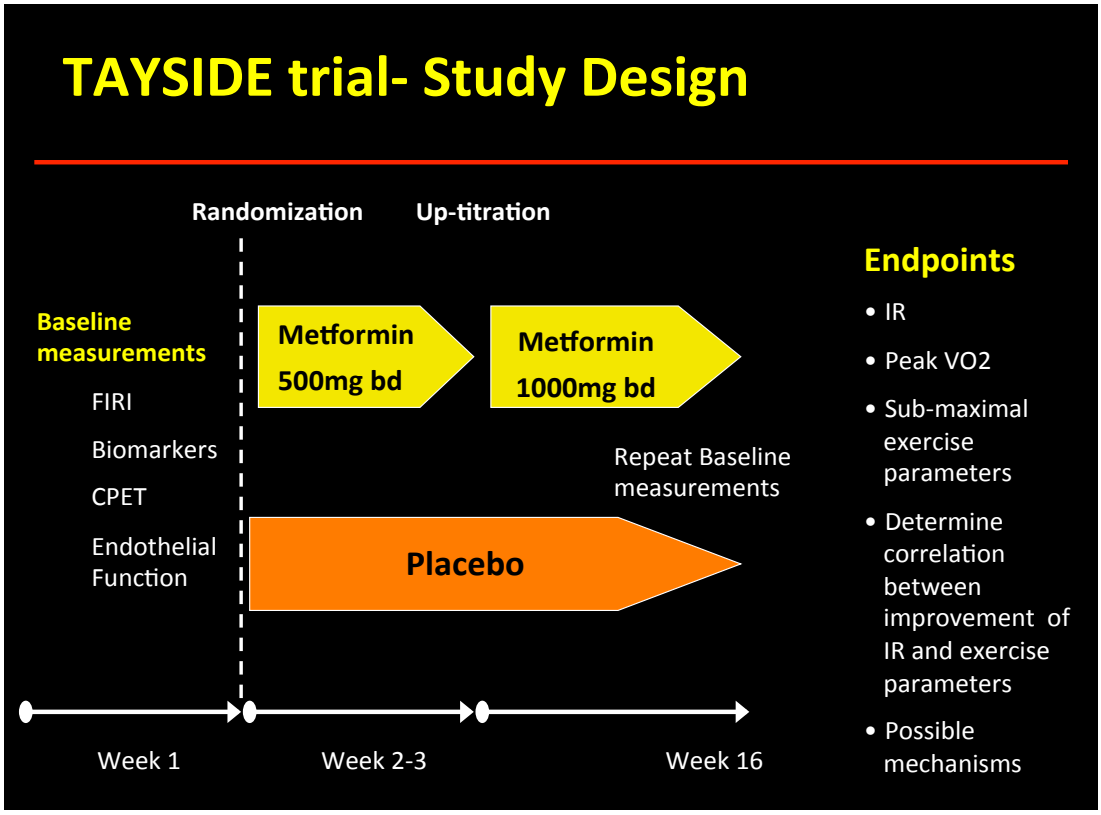


Figure 13 TAYSIDE Trial Design

CARDIOPULMONARY EXERCISE TESTING (CPET)

CPET was performed in the fasting state as previously described (188). Prior to exercise, the patient was instructed on the re-breathing technique. An incremental symptom-limited bicycle exercise testing was performed using an upright, braked cycle ergometer. After 3 minutes rest, exercise was begun at 0 Watts (W) and increased every 3 minutes by 25W until symptom-limited maximal exercise was achieved. Patients were instructed to signal approximately 1 minute before peak exercise. ECG was monitored continuously during the test. Cuff blood pressure was measured at rest and every 3 minutes. Expired gas analysis was performed continuously throughout the test with the Innocor system (Innovision A/S, Odense, Denmark). Peak VO_2 was defined as the highest value of VO_2 achieved in the final 20 seconds of exercise. VE/VCO_2 slope was calculated from the start of incremental exercise to the anaerobic threshold, by least squares linear regression. Cardiac output (CO) measurements were made at the end of the rest period and at peak exercise. VO_2 (ml/kg/min), VCO_2 (L/min), and VE (L/min) (minute ventilation) were measured on a breath-by-breath basis. CPETs were performed at visit 1 and visit 2 to achieve a variation of exercise duration of less than 15% prior to randomisation to minimise the "learning effect". If variation was more than 15%, a further CPET was repeated and the highest value of peak VO_2 was chosen to be the baseline.

ENDOTHELIAL FUNCTION

A number of non-invasive methods of assessing endothelial function are available. We have chosen Reactive Hyperaemia Peripheral Arterial Tonometry, RH-PAT (Itamar Medical Ltd. Caesarea, Israel) (Figure 14) and Flow-Mediated Dilatation (FMD) (116,189) (Figure 15) to assess vascular function in different vascular beds.

FLOW-MEDIATED DILATATION (FMD)

Endothelial function has been shown to predict future cardiovascular events (190). Flow-mediated dilatation (FMD), available since 1992, is currently viewed as the gold standard for assessing endothelial function non- invasively (109). FMD provides useful prognostic information based on the concept that direct assessment of the function of the arterial wall has more predictive power compared to assessing traditional risk factors. Table 7 summarises the studies using FMD as a prognosticator in subjects with cardiovascular disease or high risk for developing cardiovascular disease (191). FMD has been documented to correlate with invasively assessed endothelial function in the coronary arteries. (192). Endothelial function in the brachial circulation is impaired as in the coronary circulation in the setting of traditional and novel risk factors and responds to interventions known to reduce CVD risk (190) . FMD measures change in diameter of a conduit vessel following a period of ischaemia, and the brachial artery is the most commonly studied vessel. A sphygmomanometer cuff is placed on the forearm distal to the brachial artery and inflated to supra-

systolic blood pressure for 4-5 minutes and the cuff is released. The resulting reactive hyperaemia increases shear stress leading to NO release, and therefore endothelium dependent vasodilatation (184). Endothelium independent vasodilatation is assessed by response of brachial artery to sublingual GTN. FMD is widely used and correlates well with coronary vascular endothelial function (109). However FMD has several limitations, which precluded its integration into clinical practice. It is technically difficult to perform and require extensive sonographer training, together with the expense of equipment and the requirement of labor-intensive image analysis, and lack of methodological standardization that have prompted a search for techniques inherently faster and easier to perform.

First Author	Journal	N	Male, %	Age, y	Group	Follow-Up	End Points (n)	Prognostic Impact
Meyer	*J Am Coll Cardiol*	75	89	56	CHF	1.5	Progression	FMD predicts progression disease (independent)
Neunteufl	*Am J Cardiol*	73	52	51	Chest pain+CAG	5	CV events (23)	FMD predicts CV events (independent)
Gokce	*J Am Coll Cardiol*	199	82	67	PAD	1.2	CV events (35)	FMD predicts CV events (independent)
Fathi	*J Am Coll Cardiol*	444	59	59	CAD	2	CV events (119)	Low risk: FMD does not predict CV events; high risk: FMD predicts CV events (independent)
Brevetti	*Circulation*	131	90	64	PAD	1.9	CV events (39)	FMD predicts CV events (independent)
Karatzis	*Am J Cardiol*	98	100	63	NSTEMI	2	CV events (20)	FMD predicts CV events (independent)
Fischer	*European Heart Journal*	67	61	82	CHF	3.9	CV events (24)	FMD predicts CV events (independent)
Chan	*J Am Coll Cardiol*	152	56	84	CAD	2.8	CV events (22)	FMD/GTN ratio predicts CV events (independent)
Frick	*J Am Coll Cardiol*	398	100	54	Chest pain+CAG	3.3	CV events (44)	FMD does not predict CV events
Patti	*Circulation*	136	81	63	CAD+stent	0.5	Restenosis (20)	FMD predicts restenosis
Kitta	*J Am Coll Cardiol*	141	68	66	PCI	0.5	Restenosis (46)	FMD (during follow-up) predicts restenosis

CV indicates cardiovascular; CAD, coronary artery disease; PCI, percutaneous coronary intervention; CAG, coronary angiography; CHF, congestive heart failure; NSTEMI, non-ST elevation myocardial infarction; PAD, peripheral artery disease; GTN, glyceryl trinitrate.

Table 7: FMD as a prognosticator in subjects with cardiovascular disease or at high risk for cardiovascular disease. Green D et al. Hypertension 2011;57:363-369

PULSE AMPLITUDE TONOMETRY (PAT)

Digital pulse amplitude tonometry (PAT) is a new non-invasive technique to measure endothelial function. It measures volumetric changes in the fingertip, using a probe that quantifies pulse amplitude in response to reactive hyperemia using a commercially available device (EndoPAT, Itamar Medical, Ltd). Signals in the contralateral hand not experiencing hyperemia are simultaneously recorded, controlling for systemic effects. Proprietary software provides a reactive hyperemia PAT ratio in relation to the control arm that is expressed after natural log transformation owing to skewed variable distribution. The potential advantage of this technique relates to the use of an automated, computerized analysis system that minimizes operator dependency and inter-observers variability. Small-scale preliminary studies showed that PAT hyperemic responses depend on NO and were reduced in the presence of coronary artery disease or its risk factors, suggesting that clinically important group differences can be detected using this method (193).

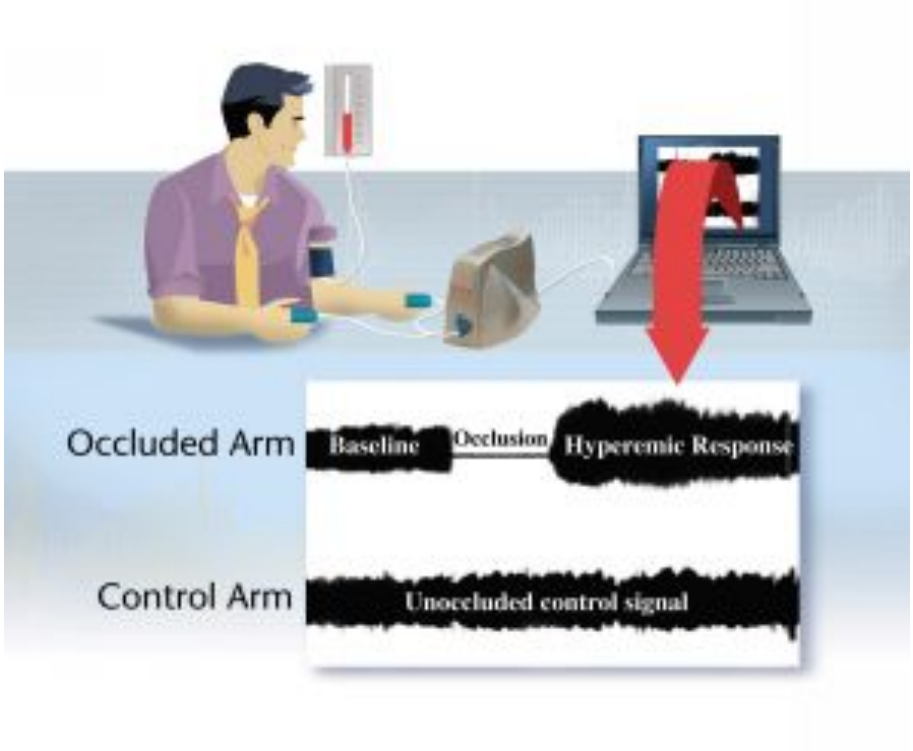
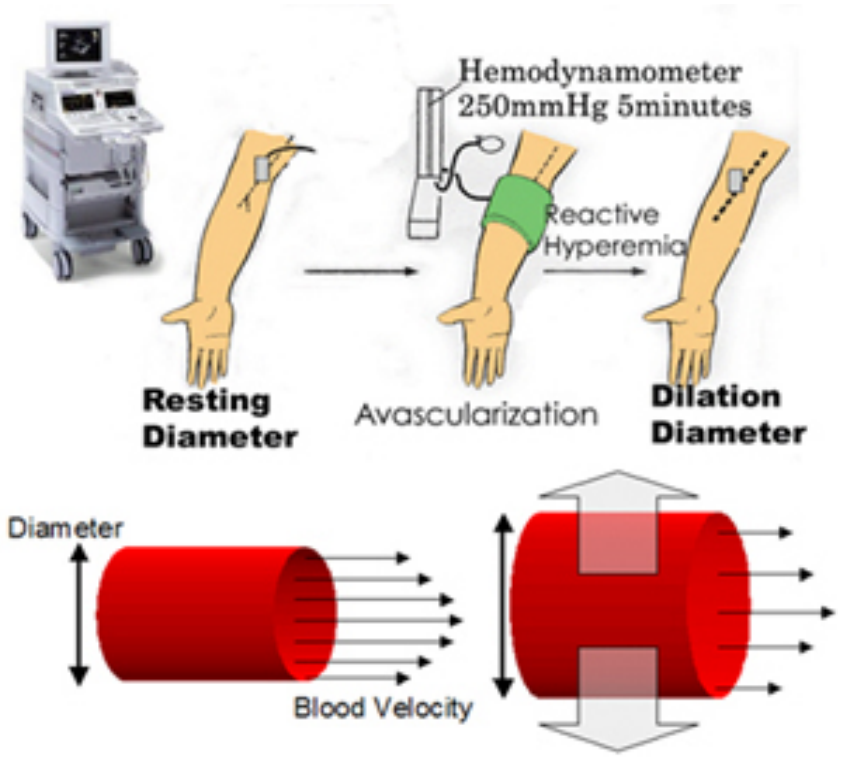
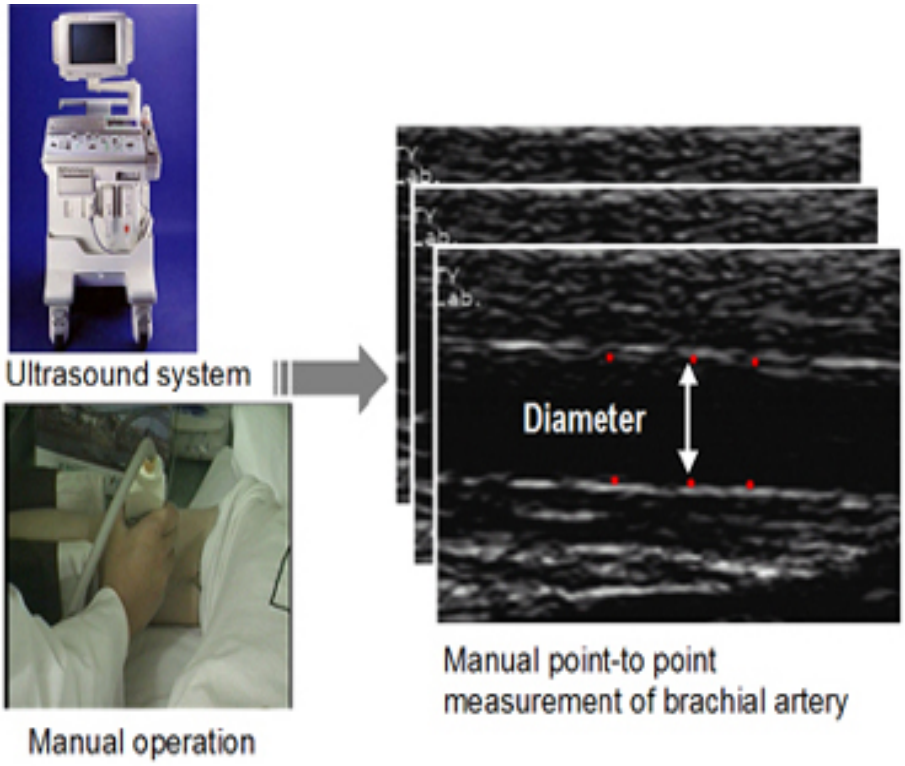


Figure 14: Reactive hyperaemic tomography (Adopted from ITamar Medical)

Figure 15: Flow mediated dilatation. (Adopted from BMPE, University of Tokyo)





ECHOCARDIOGRAPHY

Standard 2-D echo was performed in all patients. Biplane left ventricular ejection fraction (LVEF) was calculated using the Simpson's method.

6MWT WAS PERFORMED USING A STANDARDISED APPROACH OVER A 25 METERS COURSE.

MINNESOTA LIVING WITH HEART FAILURE QUESTIONNAIRE (MLHF)

This is a 21-item questionnaire, which assesses physical activity, subjective symptoms and psychosocial issues.

BIOMARKERS

Venous blood samples were obtained following a 20 min semi-recumbent rest in the fasting state at baseline, and at end of study for measurement of full blood count, renal function, glycated haemoglobin (HbA1c), glucose, lipids, lactate, insulin (INSIK-5, DiaSorin, UK), brain natriuretic peptide (BNP), adiponectin (Quantikine, R&D System, UK), leptin (Quantikine, R&D System, UK) and resistin.

SAFETY ASSESSMENTS

Safety was assessed via monitoring for adverse events (AEs), clinical examination, standard laboratory testing, ECG recordings and regular measurements of vital signs. Lactate levels were measured 2 weeks after initiation of study treatment, and at the end of study visit.

POWER CALCULATION AND STATISTICAL METHOD

We targeted 66 subjects and the power calculations were based on our previous observational study of CHF with IR with a mean peak VO_2 of 11 ml/min/kg and standard deviation of 1.8ml/min/kg, which would provide 80% power to detect a 13.5% change in peak VO_2 in the 2 groups of patients with CHF (alpha=0.05) allowing for a 10% drop out rate. Statistical analysis was performed using SPSS for Windows version 16 (SPSS Inc., Chicago, IL). Numeric values were expressed as mean ± standard deviation. An intention to treat analysis was used. The significant of differences between the two treatment groups of changes from baseline was analyzed using independent t-tests and chi-square tests. Correlations were made using Pearson product moment correlation coefficients. P value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics were well matched between the 2 study groups (Table 8). Baseline measurements of interests are shown in Table 9.

Table 8: Baseline characteristics of TAYSIDE Study

	Metformin N=39	Placebo N=23	All Patients N=62	P Value
Age	64 ± 8	68 ± 7	65 ± 8	0.063
Sex (Male/Female)	35/4	22/1	57 / 5	0.409
Systolic Blood Pressure (mmHg)	113 ± 16	114 ± 17	113 ± 16	0.761
Diastolic Blood Pressure (mmHg)	71 ± 9	73 ± 10	72 ± 9	0.563
Mean Heart Rate (bpm)	74 ± 16	71 ± 19	73 ± 16	0.597
Body Mass Index (BMI)	30 ± 5	29 ± 4	29.7 ± 4.6	0.224
LV Ejection Fraction (%)	34 ± 8	30 ± 8	33 ± 8	0.083
NYHA (I/II/III/IV)	7/29/3/0	3/17/3/0	10 / 46 / 6 / 0	0.725
Aetiology of Heart Failure				
Ischaemic	28	21	49	0.068
Non-ischaemic	11	2	13	
Past Medical History (%)				
Ischaemic Heart Disease	80	91	63	0.036
Atrial fibrillation	31	17	26	0.245
Revascularisation	33	35	34	0.944
Peripheral vascular disease	3	9	5	0.277
Hypertension	41	43	55	0.850
Chronic Obstructive Pulmonary Disease	3	13	7	0.105
Medications (%)				
Diuretics	51	52	53	0.946
Angiotension Converting Enzyme inhibitors	74	82	74	0.473
Angiotensin receptor blockers	21	14	18	0.474
Betablockers	82	91	82	0.329
Aldosterone antagonist	37	14	27	0.055
Digoxin	8	14	10	0.475
Calcium channel blocker	11	18	13	0.401
HMG Co-A reductase Inhibitors (Statin)	82	100	91	0.031
Isosorbide Mononitrate	18	36	12	0.122

Table 9: Baseline measurements of TAYSIDE Study

	Metformin N=39	**Placebo N=23**	**P Value**
Exercise Parameters			
▪ Peak VO_2 (ml/kg/min)	19.5 ±4.9	18.5 ±5.1	0.312
▪ Peak Cardiac Output (L/min)	8.3 ±2.9	7.8 ±2.6	0.631
▪ VE/VCO_2 Slope	32.9 ±15.9	32± 5.9	0.821
▪ Ventilatory Class I/II/III/IV	13/12/7/1	8/7/5/0	0.845
▪ Respiratory Gas Exchange Ratio (R)	0.9 ±0.1	0.8 ±0.1	0.088
▪ Total exercise duration (secs)	1049 ±207	940 ±288	0.091
▪ 6 Minute Walk Test (meters)	438 ±76	414±86	0.280
Endothelial Function			
▪ Reactive Hyperaemic Index (RHI)	1.8 ±0.3	1.9 ±0.6	0.264
▪ Flow Mediated Dilatation (FMD) (%)	7.1 ±3.7	5.4 ±3.7	0.119
Laboratory Parameters			
▪ Haemoglobin (g/dL)	14.8 ±1.4	14.5 ±1.3	0.361
▪ Creatinine (µmol/L)	87.8 ± 16.3	88.3 ± 19.5	0.912
▪ Glucose (mmol/L)	5.6 ± 0.6	5.3 ± 0.4	0.029
▪ Insulin (mU/L)	26.8 ± 14.3	23.2 ± 10.5	0.198
▪ Fasting Insulin Resistance Index (FIRI)	6.6 ± 3.9	6.5 ± 4.6	0.116
▪ Total Cholesterol (mmol/L)	4.3 ± 1.0	3.8 ± 0.6	0.023
▪ Adiponectin (mg/ml)	8.4 ± 6.7	8.7 ± 5.5	0.879
▪ Leptin (ng/ml)	16.6 ± 23.2	10.9 ± 6.4	0.265
▪ BNP (pg/ml)	131.7± 158.5	187.1 ± 251.3	0.362
▪ Resistin (ng/ml)	5.1 ± 2.3	4.0 ± 1.1	0.054

Changes in weight, FIRI and biomarkers

Compared to placebo, metformin resulted in a significant reduction in weight and BMI (p< 0.001, p=0.037 respectively). Metformin significantly decreased FIRI (p<0.001) and serum HbA1c (p=0.002). Serum leptin levels were significant reduced with metformin (metformin, -4.56 ± 11.0 ng/ml vs placebo, 0.58± 3.5 ng/ml, p=0.038). There was no significant change in plasma BNP with metformin (metformin, -20.2 ± 78.7 pg/ml vs placebo, 7.5 ± 131.2 pg/ml).

Maximal and Sub-maximal Exercise Parameters and 6MWT

Peak exercise parameters did not differ between treatment groups (Table 10). Compared to placebo, metformin decreased the sub-maximal parameters of VE/VCO_2 slope (from 32.9±15.9 to 28.1± 8.8, p=0.034) and ventilatory class (χ-square test, p=0.008). There was no difference in 6MWT.

Correlations between VE/VCO2 slope, Weight and FIRI

Pearson correlations and linear regression model showed that weight reduction on its own was not correlated with the reduction of VE/VCO_2 slope (p=0.801). In the metformin treated group, FIRI and serum leptin levels were significantly related to the reduction of VE/VCO_2 slope (R=0.41, Difference in FIRI: Beta: -14.07, p=0.036; Difference in leptin: Beta=0.29, p=0.023).

Symptoms

There was no significant change in NYHA functional class between the treatment groups (χ-square test, p=0.124). Although it was noted that 4 patients in the metformin arm reported an improvement in NYHA functional class with a drop of one NYHA functional class whereas 1 patient in the placebo arm had an increase in one NYHA functional class. MNLHF Questionnaire scores did not differ between the groups. Heart failure medications including diuretic dosage remained unchanged throughout the study.

Echocardiography and Endothelial Function

There was no significant change in LVEF. Changes in RH-PAT (metformin, 0.12 ± 0.4 vs. placebo, 0.06 ± 0.70) and FMD (metformin, -0.38 ± 4.46 vs. placebo, -1.74± 2.71) were not statistically significant.

Tolerability and Safety of Metformin

There were no SAEs in either treatment groups. AEs were more frequent with metformin treatment although majority of these AEs were transient, and were mild to moderate in severity. The main AEs were abdominal discomfort (16% metformin, 4% placebo, p= 0.18), diarrhoea (47% metformin, 13% placebo, p= 0.008), nausea (29% metformin, 0% placebo, p= 0.005), and anorexia (21% metformin, 0% placebo, p= 0.021). AEs led to premature discontinuation in five metformin treated patients. The average tolerable dose of metformin was 1675mg daily. Lactate levels did not differ between treatment groups and no lactic acidosis was reported.

Table 10: TAYSIDE study. Changes after 4 months of metformin treatment

	Metformin N=36	**Placebo N=22**	**P value**
Peak Exercise Parameters			
Peak VO_2 (ml/kg/min)	-0.38 ± 1.40	3.60 ± 3.90	0.08
Peak CO (L/min)	0.03 ± 3.10	-0.35 ± 2.10	0.682
Sub-maximal Exercise Parameters			
VE/VCO_2 Slope	-4.45 ± 10.72	-0.23 ± 3.54	0.034
Ventilatory Class I/II/III/IV	21/8/2/2	8/6/7/1	0.008
NYHA Functional Class I/II/III/IV	10/25/1/0	3/15/4/0	0.124
Heart Failure Questionnaires	1.58 ± 14.78	0.45 ± 8.57	0.746
6 Minute Walk Test (meters)	6 ± 40	6 ± 32	0.988
Biomarkers and laboratory parameters			
HbA1c (%)	-0.12 ± 0.19	0.08 ± 0.17	0.035
Glucose (mmol/L)	-0.36 ± 0.45	0.09 ± 0.71	0.005
Insulin (mU/L)	-6.60 ± 8.80	4.10 ± 13.10	0.000
FIRI (Log)	-1.44 ± 0.16	0.05 ± 0.18	0.000
Leptin (ng/ml)	-4.56 ± 11.0	0.58 ± 3.50	0.038
Adiponectin (mg/ml)	-0.44 ± 2.16	0.43 ± 2.54	0.168
Resistin (ng/ml)	0.01 ± 0.08	0.04 ± 0.06	0.094
BNP (pg/ml)	-20.2 ± 78.7	17.5 ± 131.2	0.184
Creatinine (μmol/L)	0.50 ± 12.46	1.41 ± 7.39	0.758
Lactate (mmol/L)	0.09 ± 0.63	0.00 ± 0.42	0.562
Cholesterol (mmol/L)	-0.32 ± 0.54	-0.01 ± 0.65	0.055
Triglyceride (mmol/L)	-0.17 ± 0.60	-0.04 ± 0.80	0.467
Weight (Kg)	-1.9 ± 2.3	1.1 ± 2.5	0.000
Body Mass Index	-2.03 ± 6.07	0.39 ± 0.89	0.037
Waist Hip Ratio	-0.08 ± 0.03	-0.49 ± 2.02	0.341
Ejection Fraction (%)	0.35 ± 5.50	-1.10 ± 4.20	0.356
Endothelial Function			
Reactive Hyperaemic Index (Endo-PAT)	0.12 ± 0.54	0.06 ± 0.70	0.743
Flow Mediated Dilatation (%)	-0.38 ± 4.36	-1.74 ± 2.71	0.201

DISCUSSION

This study had two main findings, Firstly, we showed that in patients identified to have insulin resistance and CHF, metformin treatment significantly reduced FIRI and that this treatment was associated with a weight loss of 1.9 kg. Secondly, although metformin had no effect on peak exercise parameters including peak VO_2, metformin treatment did result in a significant improvement in VE/VCO_2 slope, a pre-specified endpoint of this proof of concept study.

Although diabetes predispose to the development of CHF, CHF may also lead to the development of IR and diabetes. Previous clinical studies utilizing the hyperinsulinaemic-euglycaemic clamps have demonstrated fasting hyperinsulinaemia and insulin resistance in patients with both ischaemic and non-ischaemic CHF (174). IR is highly prevalent amongst patients with CHF. We and others have showed that close to two thirds of patients with CHF has IR determined by either FIRI or oral glucose tolerance test (116,182). IR is associated with decreased exercise capacity, endothelial dysfunction (116) and more importantly worse prognosis in CHF (2,3). The prognostic impact of IR is independent of other variables, including peak VO_2 and left ventricular ejection fraction, which may imply that IR maybe pathogenic rather than simply a marker for worsened CHF (175,194). These findings support the notion that IR may be pathophysiologically linked with CHF, and is implicated in the disease progression in CHF (195). This is likely because IR is associated with endothelial dysfunction, inflammation, increased oxidative stress, changes in

cardiac metabolism and myocardial remodelling, processes that accelerate the progression of disease in CHF (195).

If IR is important to the pathogenesis of CHF, it could be argued that therapies directed toward improving IR could be beneficial. To the best of our knowledge, this is the first proof of concept study to examine the impact of metformin on IR and exercise parameters in patients with CHF identified to have IR. In this study, we have chosen to use metformin although we recognize that the precise mechanisms of metformin's action are not entirely understood (176). However, there is increasing evidence to suggest that metformin may have cardio-protective effects in the setting of CHF that are not attributed to the glucose lowering effects alone. Recent experimental studies suggest ancillary potential mechanisms. These protective effects may be conferred via the 5'-AMP-activated protein kinase (AMPK) pathway, which is activated by metformin (165,196,197). Metformin has also been shown to improve endothelial function by increasing nitric oxide production (198). Clinical studies show that metformin may reduce plasma dipeptidyl peptidase-4 activity and increase circulating levels of glucagon-like peptide 1 (GLP-1), which is an incretin hormone that has protective effects on the heart and the vasculature (199,200). Finally, there are observational studies of patients with CHF and type 2 diabetes mellitus taking metformin that suggest a morbidity and mortality benefit (18,184). Although these data were encouraging, the main limitations of these observational studies were the potential for selection bias imposed by different therapies. What is needed are prospective placebo controlled studies such as our study.

In this study, we were interested in determining if reversing IR with metformin in patients with CHF would result in an improvement in exercise capacity. In this study, we did not observe an effect of metformin on peak exercise parameters including peak VO_2, the primary endpoint of our study. However, metformin treatment did result in VE/VCO_2 slope and ventilatory class. The VE/VCO_2 slope reduced from 32.9 to 28.1. The VE/VCO_2 slope had been reported to be a more accurate prognostic index for cardiac related mortality and hospitalization than peak VO_2 (201,202), and the ventilatory classification system has been proposed to guide therapy in patients with CHF (203). We acknowledge that there are several possible explanations for our findings. One possible explanation for the improvement in functional capacity might be the weight loss of 1.9 kg associated with metformin therapy. Studies of both diet and drug-induced weight loss have been shown to improve functional status in CHF patients (204). However, it should be noted that our regression model showed that weight reduction did not correlate with the reduction in VE/VCO_2 slope. Another consideration is that the insulin sensitizing properties of metformin might confer some beneficial effects on exercise capacity. Improving insulin sensitivity has been shown to improve exercise capacity. Regensteiner and colleagues have previously shown that improvement in insulin sensitivity with rosiglitazone resulted in a significant improvement in exercise capacity and peak VO_2 in diabetic individuals (205). In this regard, we found that with metformin treatment, reduction of FIRI and serum leptin level was significantly correlated with the reduction of VE/VCO_2 slope. Doehner et al had previously demonstrated that hyperleptinaemia is an independent predictor of IR in patients with CHF, and it may play an important role in

energy metabolism in these patients (63). Reduction of serum leptin levels have been previously reported following chronic metformin therapy (206), and might be due to a direct effect of metformin on leptin secretion (207). Recent studies have shown the significance of adipocytokines modulation in HF patients; and high levels of adiponectin were associated with adverse outcomes in CHF (75,76). There were no significant change of adiponectin levels in our study (p=0.168). However, we did notice that patients in the metformin treated group have decreased adiponectin level whereas adiponectin levels were higher in the placebo group. Therefore, the improvement of sub-maximal exercise capacity in our cohort may in part be due to an improvement of IR and the reduction of serum leptin level. A third consideration is metformin's ability to activate AMPK, which is expressed in various tissues including the skeletal muscle, myocardium as well as the vascular endothelium (165). Therefore, activation of AMPK could impact on central as well as peripheral haemodynamic mechanisms, which in turn leads to changes in VE/VCO_2 slope and hence, ventilatory class. Improvement of myocardial substrate utilization and glucose uptake through activation of AMPK may improve myocardial contraction and increase cardiac output (196). In animal models of heart failure, metformin has been shown to activate AMPK and improve left ventricular function and to attenuate oxidative stress-induced cardiomyocyte apoptosis, resulting in improved survival (197). However, we did not observe an effect of metformin on echo derived LVEF or CO at rest and during peak exercise. With respect to peripherally mediated mechanisms, an effect of metformin on exercising skeletal muscles could account for the improvement in VE/VCO_2 slope. Alterations in skeletal muscle energy metabolism, IR and

functional adiponectin resistance have been reported, and linked to exercise intolerance in patients with CHF (208). A study of the effect of metformin on skeletal muscle enzyme activities in our subjects would be of interest. In our original proposal, we had planned to do this but this invasive procedure was offered as an option and no patient consented to the procedure. We did not see an effect of metformin on endothelial function. Obviously any evidence that metformin improves exercise capacity through central cardiac or peripheral mechanisms in patients with CHF must remain speculative and cannot be inferred directly from this study. Clearly, further studies are required to define the mechanisms underlying these effects of metformin in CHF.

In this study, we did not record any incidence of lactic acidosis and SAEs. The most common AE was diarrhoea, which is well described with metformin use. The 2 kg reduction in weight is consistent with findings of previous studies of metformin when used in non-diabetic populations (209).

LIMITATIONS OF STUDY

In order to comply with our strict inclusion criteria, patients recruited had to be able to perform repeated CPETs. We believe that these strict inclusion criteria might have resulted in us recruiting a cohort of patients with milder CHF as our patients had a higher peak VO_2 (19 ml/kg/min) than the peak VO_2 (11 ml/kg/min) that we based our power calculations on. This might explain why we did not observe an effect of metformin on the primary endpoint of the study, peak VO_2. Even though this is a randomised controlled study, there were inevitable potential confounding factors noted although they were not

statistically significant between groups. Patients in the metformin group were somewhat younger, LVEF was higher and with more non-ischaemic aetiology of heart failure. Therefore, they may have been able to exercise more within the period of the study.

CONCLUSIONS

This proof of concept study has shown that in non-diabetic CHF patients identified to have IR, treatment with metformin significantly improved IR, VE/VCO_2 slope and resulted in significant weight loss, but did not improve peak VO_2, the primary endpoint of the study. Although the improvement of VE/VCO_2 slope was correlated with the improvement of IR, we were not able to ascertain if this was the cause of improvement of sub-maximal exercise performance owing to the complexity of action of metformin. Our findings are however hypothesis generating, and further studies are clearly required to determine the effects of metformin on exercise performance in patients with CHF.

CHAPTER 7: THE FUTURE INSULIN RESISTANCE MODULATORS: AMP-ACTIVATED PROTEIN KINASE ACTIVATORS

ABSTRACT

The 5'-AMP-activated protein kinase (AMPK) is a heterotrimeric enzyme that is expressed in many tissues including the heart and vasculature, and plays a central role in the regulation of energy homeostasis. It is activated in response to stresses that lead to an increase in the cellular AMP: ATP ratio caused either by inhibition of ATP production (i.e. anoxia, ischaemia) or by accelerating ATP consumption (i.e. muscle contraction, fasting). In the heart, AMPK activity increases during ischaemia and functions to sustain ATP, cardiac function and myocardial viability. There is increasing evidence that AMPK is implicated in the pathophysiology of cardiovascular and metabolic diseases. A principle mode of AMPK activation is phosphorylation by upstream kinases (e.g. LKB1, calcium calmodulin dependent protein kinase), which leads to direct effects on tissues and phosphorylation of various downstream kinases (i.e. eEF2 kinase, p70S6). These upstream and downstream kinases of AMPK have fundamental roles in glucose metabolism, fatty acid oxidation, protein synthesis and tumour suppression; consequently, they have been implicated in cardiac ischaemia, arrhythmias and hypertrophy. Recent mechanistic studies have shown that AMPK has an important role in the mechanism of action of metformin, thiazolidinediones and statins. Increased understanding of the beneficial effects

of AMPK activation provides the rationale for targeting AMPK in the development of new therapeutic strategies for cardio-metabolic disease.

INTRODUCTION

The prevalence of cardiometabolic diseases is reaching epidemic proportions in industrialized nations and in developing countries (210-212). Despite aggressive treatment of the individual cardiometabolic risk factors, death from cardiometabolic conditions remains unacceptably high. Therefore, there is an urgent need to identify new strategies for treating and preventing cardiometabolic diseases. In this respect, the AMP-activated protein kinase (AMPK) pathway has become the focus of a great deal of attention as a novel therapeutic target in cardiometabolic disease, because it has been demonstrated to mediate, at least in part, the effects of a number of physiological and pharmacological factors that exert beneficial effects on the vasculature and the heart. AMPK has several important metabolic effects including increasing muscle glucose uptake (213) (214), and ameliorating insulin resistance (215). It regulates cardiac muscle glucose and lipid metabolism both directly and indirectly in order to provide ATP in response to energy depletion (specifically a rise in the AMP: ATP ratio). AMPK activity can also be modulated by hormones and adipocytokines, which may have protective effects against cardiovascular disease. AMPK has also been shown to regulate transcription of genes involved in lipid and glucose metabolism (216,217). Dysregulation of this process (e.g. in obesity) can lead to the development of insulin resistance and dyslipidaemia, both of which are major risks factors for

CVD. Thus, the identification of a compound that specifically and safely activates the AMPK pathway might contribute significantly to the treatment, management and even prevention of CVD. We aim to discuss the direct and indirect role of AMPK in normal cardiac physiology and in cardiometabolic disease, and therapeutic strategies in modulating APMK activity.

STRUCTURE AND REGULATION OF AMPK

Understanding of the role of AMPK in key physiological pathways has increased several folds in recent years. Its discovery can be traced back to two independent findings reported in 1973 (218) which observed that crude preparations of acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methyl (HMG)-CoA reductase became inactivated when incubated with ATP. Both groups predicted that the effects were due to phosphorylation of the enzyme by an endogenous protein kinase that contaminated their preparations. It was subsequently shown that this protein kinase was itself activated by phosphorylation by an upstream kinase (219). In 1987, Hardie *et al* made the discovery that the inactivation of ACC and HMG-CoA reductase were both catalyzed by a single protein kinase (220). As it became clear that it was a true multi-substrate kinase, they renamed it AMP-activated protein kinase after its allosteric activator, 5'-AMP (221). Hardie et al have described AMPK as a 'fuel gauge' and 'guardian of energy status', implying the fundamental role of AMPK in energy metabolism and maintaining body energy balance. AMPK is a heterotrimetric enzyme complex which consists of α, β, and γ subunits, each of which has two or more isoforms that are encoded by distinct genes and are

differentially expressed in various tissues (222). The α subunit contains the catalytic domain, including the important regulatory Thr-172 residue, which is phosphorylated by upstream kinase. The β subunit has a glycogen-binding C-terminal domains that are sufficient on their own to form a complex with α and γ subunits. High cellular glycogen content exerts an inhibitory effect on AMPK through interaction with the β subunit in skeletal muscle, although the exact mechanism is unknown (223). The γ subunit of AMPK was first recognized by Bateman (224) and contains 4 repeats, forming two domains. Each of these domains binds one molecule of AMP or ATP ion in a mutually exclusive manner (225), consistent with the findings that high concentrations of ATP antagonize activation of AMPK by AMP.

For many years, the upstream kinases(s) that phosphorylate Thr-172 on the α subunit of AMPK remained unidentified. In recent years, it has been established that the major upstream kinase in mammalian cells is a complex of protein kinase LKB1 and two accessory subunits, STRAD and MO25 (226-228). LKB1 also acts as an upstream kinase of at least 12 other AMPK-related kinases (229,230). It has also been found to be a tumour suppressor and was identified in humans as a gene carrying an autosomal dominant mutation in Peutz-Jeghers syndrome (228,231). The STRAD subunit is essential for the ability of the LKB1 complex to phosphorylate Thr-172 on AMPK (227). Besides LKB1, STRAD and MO25, AMPK can also be activated by an LKB1-independent mechanism involving Ca^{2+}/calmodulin dependent protein kinases.

AMPK exerts its metabolic effects through its interactions with various metabolic pathways. Activation of these metabolic pathways via AMPK

activation leads to remodeling of various components of metabolic syndrome (232) (Figure 16). AMPK plays a major role in providing ATP in the midst of energy depletion via its interactions with various metabolic pathways (Figure 17). Furthermore, AMPK also has direct and indirect effects on cardiovascular system. The understanding of such effects provide the rationale of targeting AMPK as a new therapeutic modality for the treatment and prevention of CVD.

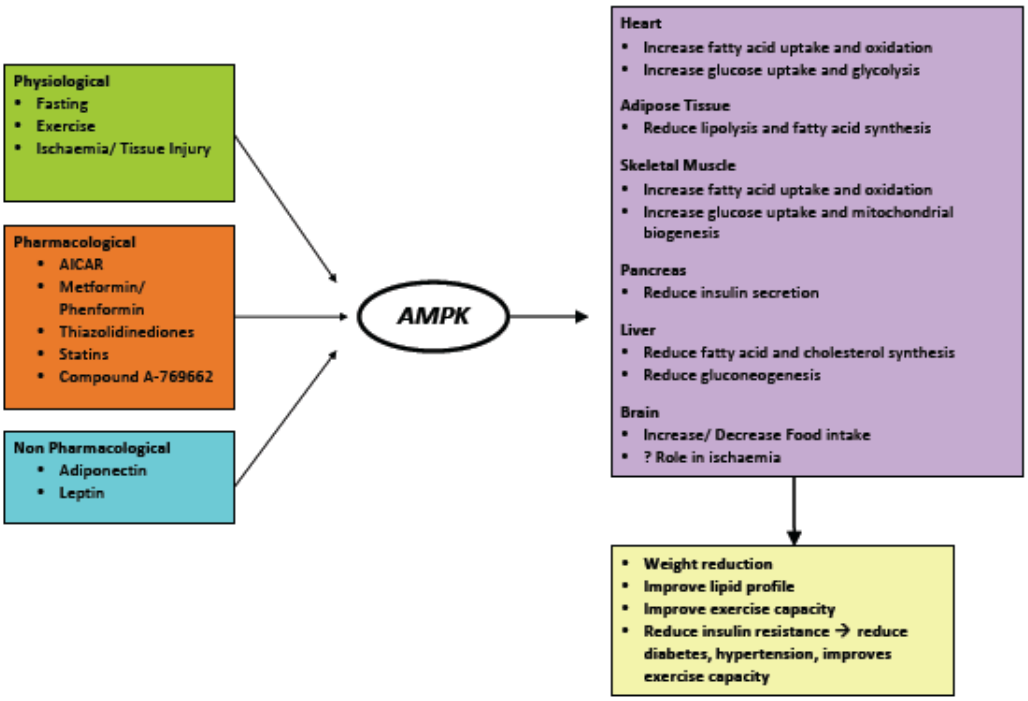


Figure 16: Activation of various metabolic pathways via AMPK activation leads to remodelling of various components of metabolic syndrome

AMPK: DIRECT EFFECTS ON CARDIOVASCULAR SYSTEM

Congestive cardiac failure, left ventricular hypertrophy, myocardial ischaemia and diabetic cardiomyopathy are all associated with disturbance of cardiac energy homeostasis. In these pathological states, AMPK activity is up regulated in response to increased AMP/ATP ratio (energy-depleted state). AMPK switches on energy generating pathways to increase cardiac myocytes fatty acid uptake (233), and increases glucose uptake by increasing translocation of GLUT-4 in an PI3-K independent manner (214) while also enhancing glycolysis via PFK-2 activation (234). At the same time, AMPK turns off protein synthesis pathways by activating eEF2 kinase, resulting in the phosphorylation and inactivation of eEF2 and by decreasing Thr-389 phosphorylation of p70 ribosomal protein S6 kinase (p70S6K), another important kinase which is involved in protein synthesis (235) via mammalian target of rapamycin (mTOR) inhibition (235,236) (See Figure 17).

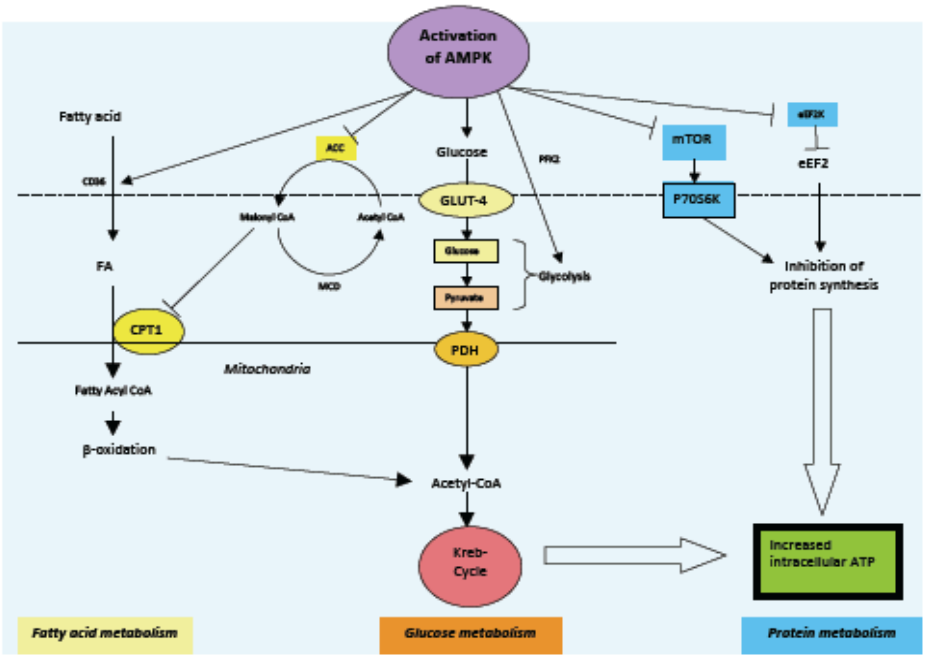


Figure 17: AMPK activation leads to activation of different metabolic pathways

AMPK plays an important role in whole body energy homeostasis. It regulates and interacts with different key metabolic pathways. Activation of AMPK secondary to change of AMP:ATP ratio or activation by upstream kinases such as CAMKK, LKB1 leads to leads to switching on energy production pathways such as glucose and lipids metabolism and turning off energy metabolic process such as protein synthesis, which is not required for immediate cell survival.

Fatty Acid metabolism. AMPK activation leads to increased translocation of CD36, a fatty acid transport protein. It increases fatty acid into cells and subsequent uptake into mitochondria for β-oxidation. Carnitine palmitoyl transferase (CPT-1) inhibits fatty acid influx and acts as a gatekeeper for mitochondrial uptake of fatty acid. Activation of AMPK leads to inhibition of acetyl CoA carboxylase (ACC2 isoform), which normally converts acetyl CoA to malonyl CoA. The inhibitory effect of malonyl CoA of CPT-1 is hence removed, leading to unopposed intake of fatty acid into mitochondria. Furthermore, phosphorylating and inactivation of the ACC1 isoform of ACC by AMPK activation reduces fatty acid synthesis and turning off expression of lipogenic genes such as fatty acid synthase.

Glucose metabolism. Activation of AMPK increases translocation and retention of glucose transporter-4 (GLUT-4) in the plasma membrane as well as increased transcription of GLUT-4 gene, leading to increased glucose uptake. It also enhances glycolysis via activation and phosphorylation of phosphofructokinase (PFK2).

Protein metabolism. p70 ribosomal protein kinase 6 (p70S6K) is a one of the key kinase involved in protein synthesis. mTOR activates p70S6K and leads to increased protein synthesis. When AMPK is activated, the activation of p70S6K is blocked as a result of inhibition of mTOR. Activation of AMPK also results in phosphorylation and inactivation of eEF2, subsequent inhibition of protein synthesis.

(A) AMPK AND CARDIAC ISCHAEMIA

During cardiac ischaemia, the AMP/ATP ratio is increased as a result of decreased oxidative metabolism of both free fatty acids and glucose due to diminished oxygen supply in the face of increased glycolytic ATP production and glucose transport (237). Russell et al have shown that AMPK activation using 5' aminoimidazole-4-carboxamide-1-β-4-ribofuranoside (AICAR) in an *in vitro* rat model increased translocation of glucose transporters (i.e. GLUT -4) into the sarcolemma, and hence increased glucose uptake(214). Furthermore, AMPK also phosphorylates and activates phosphofructokinase (PFK-2), leading to the production of fructose 2,6-bisphosphate, a potent stimulator of glycolysis. AMPK may be necessary for adiponectin to exert its cardio-protective effect against ischaemia-perfusion injury (238). Both the α1 and α2 subunits of AMPK are activated during myocardial ischaemia, with α 2 activated to a greater extent (180,239). Previous studies in transgenic mice have shown that decreased α 2 activities resulted in reduced cardiac glucose uptake following ischaemia (240) and impaired recovery of left ventricular systolic function (180). Additionally, in transgenic mice expressing a kinase dead (KD) form of the enzyme, phosphocreatinine was also lower after reperfusion (180). These observations suggested that activation of AMPK following ischaemia has a cardio-protective effect, and results in lesser cardiac injury and faster recovery. Calvert et al have also shown that activation of AMPK with metformin resulted in decreased myocardial injury in both diabetic and non-diabetic mice (241). This may be a result of deriving ATP from more energy efficient glucose

metabolism from increased AMPK-mediated glucose uptake and glycolytic flux in the face of oxygen deprivation (242,243).

However, ischaemic-induced activation of AMPK may be detrimental to the ischaemic heart, as suggested by Dyck and Lopaschuk (244). During ischaemia, circulating fatty acid levels are elevated (245), which may be detrimental to the ischaemic heart (246,247). Activation of AMPK leads to increased fatty acid uptake and inhibition of malonyl-CoA, a potent endogenous inhibitor of mitochondrial fatty acid uptake. This results in accelerated mitochondrial fatty acid uptake and hence increased mitochondrial acetyl CoA production from β-oxidation. High level of acetyl CoA has an inhibitory effect on pyruvate dehydrogenase (PDH), reducing the amount of pyruvate being converted into acetyl-CoA, hence reduced glucose oxidation (the exact mechanisms remain undefined). The proposed mechanisms of these detrimental effects of high circulating fatty acids include: (1) accumulation of toxic intermediates of fatty acid oxidation such as long chain acyl-CoA thioesters and long chain acylcarnitines (246), (2) inhibition of glucose oxidation via inhibition of PDH complex by fatty-acid-derived acetyl CoA, (3) accumulation of glycolytic by-products such as protons and lactate. These valuable observations have affirmed the role of AMPK in cardiac ischaemia and implicated a potential role for therapeutic targeting in the treatment of myocardial ischaemia and infarction.

(B) AMPK AND CARDIAC ARRHYTHMIAS

Mutations of the γ2-subunit of the AMPK have also been shown to contribute to glycogen storage disease and Wolff-Parkinson-White syndrome (248). Gollob et al identified in 2001 a mutation (Arg531Gly) in the AMP-activated protein kinase (AMPK) γ2 subunit (PRKAG2 gene) to be responsible for Wolff-Parkinson-White Syndrome and early onset of atrial fibrillation and conduction disease (248). Using a transgenic model targeting the murine gene, Davies et al demonstrated striking cardiac manifestations such as hypertrophy, impaired contractile function, electrical conduction abnormalities, and marked glycogen accumulation (249). Furthermore, Sidhu et al have identified a distinct atrial ventricular accessory pathway and prolonged QRS duration on electrocardiography in this transgenic mice model (250). However, the effects of the mutations described in this gene on the overall activity of AMPK vary in the different experimental models (251,252). It is still uncertain whether these cardiac manifestations are the result of disease-causing mutations *per se* or secondary to glycogen deposition. Murphy el al postulated that the manifestations of AMP kinase disease might be due to defects in energy utilization or in specific cellular substrates, rather than mere passive deposition of glycogen (253). Nonetheless, these data illustrate an important role for AMPK in cardiac hypertrophy and arrhythmias.

(c) AMPK AND CARDIAC HYPERTROPHY, CELL GROWTH AND GENE TRANSCRIPTION

AMPK may play a further role in the regulation of normal cardiac cell growth (180,240) and energy regulation in the hypertrophied heart (254) via its effects on protein synthesis (255,256). γ2 mutations not only cause glycogen overload in the heart and the Wolff-Parkinson-White syndrome, but also hypertrophy and heart failure (248,257-259). Severity of the defect also correlates with severity of the disease. Eukaryotic elongation factor-2 (eEF2) is the main mediator of the translocation step in protein synthesis and is inhibited through phosphorylation of eEF2 kinase. p70S6 kinase regulates protein synthesis through the same pathway or via phosphorylation of ribosomal protein S6. Chan et al have shown that AMPK not only regulates eEF2 kinase, but also exerts effects on protein synthesis via the mammalian target of rapamycin (mTOR) pathway, ultimately leading to inhibition of p70S6 (256). Furthermore, Chan et al have shown that activation of AMPK using metformin and AICAR results in inhibition of protein synthesis, and is associated with prevention and regression of cardiac hypertrophy. However, studies in transgenic mice have shown that elevated AMPK activity is associated with accumulation of large amount of glycogen, leading to dramatic left ventricular hypertrophy and arrhythmias (254,260). It remains uncertain therefore whether AMPK activation in the hypertrophied heart is beneficial (256,261) or deleterious and further studies are required.

(D) AMPK AND VASCULAR AND ENDOTHELIAL FUNCTION

AMPK also plays an important role in the regulation of vascular function and structure. It activates endothelial nitric oxide synthase (eNOS) in endothelial cells and cardiac myocytes by phosphorylation at Ser-1177 (human sequence) (262,263). eNOS activation leads to augmentation of vascular tone, platelet aggregation, leukocyte adherence and vascular smooth muscle proliferation (264).

Using a diabetic rat model, Suzuki et al has shown that activation of AMPK using a cyclic AMP (cAMP) phosphodiesterase inhibitor, cilostazol, restores endothelial function independently of cAMP (265). Administration of cilostazol leads to phosphorylation of AMPK and subsequent phosphorylation of eNOS and increased nitric oxide (NO) production. Other AMPK activators, 5-aminoimidazole-4-carboxamide riboside (AICAR) (266), metformin (267) and rosiglitazone (268) have all been shown to increase NO production in human aortic endothelial cells via the AMPK pathway. Additionally, AMPK also appears to have a role in angiogenesis, promoting the action of the HIF-1$^{\alpha}$/ vascular endothelial growth factor VEGF pathway (269) (270), and inhibiting angiotensin II-induced smooth muscle cell proliferation (271). Furthermore, activation of AMPK activation using AICAR has been shown to inhibit palmitate-induced endothelial cells apoptosis through suppression of reactive oxygen species (272). It is clear that AMPK plays a central role in vascular biology.

AMPK: INDIRECT EFFECTS ON CARDIOVASCULAR SYSTEM

Recent data have shown that levels of adipocytokines such as adiponectin and leptin correlate with the development of different components of metabolic syndrome (273). AMPK has been suggested to play a role in mediating the metabolic and vascular effects of the key adipocytokines (274,275).

(A) AMPK AND LEPTIN

Leptin is an adipocyte-secreted hormone that plays a pivotal role in the regulation of food intake, energy expenditure, body weight, and neuroendocrine function (276). Leptin stimulates fatty acid oxidation (58), and glucose uptake (59), and prevents lipid accumulation out with adipose tissue, preventing lipotoxicity (60). Deposition of ectopic fat in pancreatic beta cells, myocardium, and skeletal muscle contributes to the pathogenesis of type 2 diabetes mellitus, cardiomyopathy, and insulin resistance respectively. Leptin is known to exert effects via the AMPK pathway, stimulating phosphorylation and activation of the $\alpha 2$ catalytic subunit of AMPK selectively in skeletal muscle (58). Leptin also suppresses ACC2 activity, thereby stimulating fatty acid oxidation in muscle. AMPK also inhibits lipogenesis and ectopic fat deposition in the liver (277). AMPK is also a key regulator of leptin action in the hypothalamus and a “master regulator” of food intake. Minokoshi et al have shown that inhibition of AMPK

activity by leptin specifically in the arcuate and paraventricular nuclei is essential for its anorexigenic and weight loss effects (278).

(B) AMPK AND ADIPONECTIN

Adiponectin, an adipose-specific protein present in high concentrations in the circulation, was discovered in 1996. It possesses anti-atherogenic, insulin-sensitizing and anti-inflammatory properties. Yamauchi et al have shown that adiponectin stimulates glucose utilization and fatty acid oxidation via the AMPK pathway (274). Furthermore, adiponectin has been shown to reduce infarct size, improve left ventricular function and remodelling, and increase coronary flow during reperfusion in animal models. The underlying mechanisms are thought to be phosphorylation of eNOS, AMPK Thr 172 and Akt Ser 473 (279). Adiponectin deficient mice have been shown to have progressive cardiac remodelling in a pressure overloaded condition due to reduced AMPK signalling and worsening insulin resistance (280). Therefore, the AMPK pathway is not only critical for the metabolic- and insulin sensitizing actions of adiponectin, but also its cardio-protective effects in myocardial ischaemia and reperfusion.

AMPK ACTIVATORS: PHARMACOLOGICAL TOOLS AND THERAPEUTIC POTENTIAL

As we have seen, AMPK is a pivotal enzyme that regulates diverse signals in metabolic pathways and has direct and indirect effects on the heart and vasculature. AMPK activation has not only been shown to alleviate various components of the metabolic syndrome, but may also improve left ventricular hypertrophy and reduce cardiac injury in ischaemia. AMPK is also a key mediator of the anti-atherosclerotic and insulin-sensitizing effects of adiponectin. Therefore, it is clearly an attractive therapeutic target in cardio-metabolic disease. A number of AMPK activators are available as pharmacological tools and some are in clinical use (Table 11).

(1) 5-AMINOIMIDAZOLE-4-CARBOXAMIDE RIBOSIDE (AICAR)

AICAR is an adenosine analogue, which activates AMPK through direct binding followed by allosteric modification. It is initially taken up by adenosine transporters and subsequently phosphorylated to 5-aminoimidazole-4-carboxamide-1-ß-D-ribofuranotide (ZMP) within the cell, which mimics AMP in AMPK signalling (281). AICAR was first developed to block adenosine reuptake in the ischaemic heart, promoting stimulation of adenosine membrane receptors. In 1997, treatment with acadesine (AICAR) before and during surgery was showed to reduce early cardiac death, myocardial infarction, and

combined adverse cardiovascular outcomes (282), although the mode of action via AMPK was not fully appreciated at that time.

AICAR is now widely used in the laboratory setting, particularly in experiments relating to glucose metabolism, insulin signalling pathways and lipid metabolism. In recent years, AICAR has been shown to reverse various aspects of metabolic syndrome in animal models (232,283-285) and healthy human subjects (286)(Table 12). AICAR has also been shown to stimulates adiponectin and inhibit cytokines such as TNF-α and IL-6, which have been implicated in the development of obesity-induced insulin resistance (287-290). Unfortunately, AICAR is far from an ideal activator of the AMPK pathway in the clinical settings because of its short half-life, requirement for intravenous infusion and variable effectiveness. It also causes bradycardia and can lead to hypoglycaemia when administered intravenously. Therefore, there is great interest in developing a more potent, safer and more specific activator.

(2) METFORMIN

Metformin has been used to treat diabetes for more than 50 years and is associated in observational studies with reduced mortality and improved outcomes in patients with chronic heart failure (28,146). It is the preferred anti-diabetic medication for obese patients with Type 2 diabetes mellitus because of its property to stabilize weight and reduce cardiovascular events when used as monotherapy (291). Recent clinical studies have shown that the effects of metformin may go beyond improving glycated haemoglobin and may include reductions in cardiovascular endpoints in Type 2 diabetes mellitus and heart

failure. This wide spectrum of cardiovascular protective effects may be attributable to its activation of AMPK and its downstream pathways.

Metformin has been shown to activate AMPK in myocytes (292-294), hepatocytes (295) and skeletal muscle cells (295). Metformin decreases hepatic glucose production and increases skeletal muscle glucose disposal. Therapeutic doses of metformin have been shown to increase AMPK $\alpha 2$ activity in human skeletal muscle with an associated increase in phosphorylation of AMPK on Thr172 and decreased ACC2 activity (296). Metformin can also up-regulate eNOS and increases nitric oxide bioactivity via AMPK activation (297). Furthermore, AMPK activation by metformin enhances fatty acid oxidation, which leads to alleviation of endothelial lipotoxicity and improved endothelial function (298). Moreover, metformin has also been shown to have anti-cancer effects in recent study via its indirect AMPK activation (299). However, the precise mechanisms of how metformin activates AMPK are still poorly understood.

Even though metformin is regarded as an AMPK activator, it has not been shown to bind directly to AMPK; neither does it regulate its own phosphorylation and dephosphorylation in cell-free assays (300). One hypothesis is that it activates AMPK by inhibiting complex I of the respiratory chain, which subsequently causes a rise in the AMP: ATP ratio (301,302). In fact, inhibition of the respiratory chain in the intestinal mucosa may account for the gastrointestinal side effects of the drug and this property may account for the propensity of its predecessor biguanides phenformin to cause lactic acidosis. Metformin is transported into intestinal cells mainly by the organic cation

transporter OCT-1, but phenformin penetrates cell membranes without active transport. Recent identification of polymorphisms in genes encoding cation transporters proteins may ultimately explain differences in tolerance and response to metformin (303). Interestingly, there are also studies suggesting that AMPK can be activated by metformin without changes of AMP/ATP ratio (300,304) and metformin can also exert its beneficial metabolic effects on cardiac myocytes in an AMPK-independent manner (305).

However, we should be mindful that extra caution is required if we are to use these results to extrapolate to the effects of metformin on AMPK. Firstly, variable doses of metformin have been used in these studies. The plasma metformin concentration in clinical use is usually around 10 μM (140) whereas the doses used in vivo and in vitro experiments are consistently higher, in the range of 1-10mM (Table 13). Saeedi et al has shown that lower doses of metformin (i.e. 2mM) failed to activate AMPK and cause no changes of energetic state. On the contrary, Hardie et al have shown that lower doses of metformin can actually produce AMPK activation without significant change of cellular AMP: ATP ratio (300). Other research groups reported that AMPK activation required higher doses of metformin (i.e. 5-10mM) (292,294) (see table 13). They suggested that higher doses of metformin are required to cause changes in the energetic state and hence subsequent AMPK activation. However, these diversified results may be the result of different exposure time of metformin. For instance, Yang et al has shown that lower dose of MF (1mM) activated AMPK and increased cardiac myocytes glucose uptake after a prolonged exposure of 18 hours (293). On the other hand, Bertrand et al had shown that

short exposure (4 hours) of metformin could result in AMPK activation if much higher doses of metformin were used (5-10mM) (306). Therefore, AMPK can be activated by metformin in a time and concentration dependent manner. Clearly further studies are required to determine the time and concentration of metformin, which will result in the maximal beneficial effects of AMPK activation without intolerable side effects.

Table 11: Different "AMPK activators" and their limitations in clinical use

AMPK activators	Possible mechanisms of AMPK activation	Activations of other pathways	Limitations
AICAR	▪ Direct activation followed by allosteric modification	▪ Stimulate adiponectin release ▪ Inhibit cytokines such as TNF-α and IL-6 ▪ Please refer to table 2	▪ Short half life ▪ Variable effectiveness ▪ Only intravenous forms available ▪ May cause bradycardia and significant hypoglycaemia
Metformin (MF)	▪ Indirect activation ▪ Via alteration of AMP:ATP ratio as a results of inhibition of complex I in the respiratory chain ▪ Other unknown mechanisms ▪ Please refer to table 2	▪ Anti-cancer effects via its effects on p53 ▪ Up-regulate eNOS and increases nitric oxide bioactivity ▪ Enhances fatty acid oxidation which leads to alleviation of endothelial lipotoxicity ▪ Please refer to table 3	▪ Indirect AMPK activation ▪ Doses and duration of MF required for AMPK activation not determined ▪ Higher doses of MF results in intolerable gastrointestinal side effects
TZDs	▪ Indirect activation ▪ Via alteration of AMP:ATP ratio, possibly similar to MF ▪ Via adiponectin	▪ Anti-atherosclerotic and anti-inflammatory effects via adiponectin ▪ Effects on mitochondrial biogenesis ▪ Exerts anti-oxidative effects by inhibiting PKC via AMPK activation	▪ Indirect inhibition ▪ Risk of developing fluid retention ▪ Risk of developing cardiovascular events yet to be determined
Statins	▪ Indirect activation ▪ Does not alter AMP:ATP ratio ▪ Other unknown mechanisms	▪ HMG-CoA reductase inhibition ▪ Activation of AMPK-eNOS-ACC	▪ Doses required for AMPK activation in human still to be determined
Compound A-769662	▪ Direct activation	▪ Increased fatty acid oxidation ▪ Decreased plasma and liver triglyceride level ▪ Inhibit fatty acid synthesis ▪ Stabilize weight	▪ Poor oral bioavailability ▪ Data on long term AMPK activation are awaited

Table 12: Various Studies on AMPK activation using AICAR and their major findings

Study	Type of Subjects	Dosage	Duration	Major findings
Iglesias M A, Diabetes 2002	Insulin resistant (IR) high-fat-fed rats	Subcutaneous injection of 250mg/kg	24 hour	• Enhanced whole body, muscle and liver insulin action • Reduced hepatic glucose output
Buhl E S Diabetes 2002	Obese Zucker rats exhibiting IR, hyperlipidaemia and hypertension	Subcutaneous injection of 0.5mg/g	7 weeks	• Reduced plasma triglyceride, free fatty acids, increased HDL • Lower systolic blood pressure • Normalised oral glucose tolerance test and reduced fasting glucose and insulin • Showing tendency toward decreased intra-abdominal fat content
Bergeron R Diabetes 2001	Obese Zucker rats	Bolus 100mg/kg Constant infusion 10mg/kg/min	60 mins	• Increased glucose transport in red gastrocnemius muscle whereas insulin showed no effects • Suppression of endogenous glucose production and lipolysis
Song X. M Diabetologia 2002	Ob/Ob Mice	Subcutaneous 1mg/g of body weight	7 days	• Corrected hyperglycaemia, improved glucose tolerance, and increased GLUT4 and hexokinase II protein expression in skeletal muscle
Cuthbertson, D. J. Diabetes 2007	Healthy men	Intravenous infusion at 10mg/kg/hour	9 hours	• Increased human skeletal muscle 2-deoxyglucose uptake and whole-body glucose disposal.

Table 13: Recent studies of AMPK activation using metformin and their major findings

Study	Aims (A) and Subjects(S)	Dosage	Key findings	Clinical application
Calvert JW et al Diabetes 2008	A: Examine the cardioprotective effects of MF S: Murine models	125 mcg/kg vs saline (286 fold lower than maximum antihyperglycaemic dose)	▪ Reduction of myocardial injury in both diabetic and non diabetic mice ▪ Increased AMPK activity and eNOS phophorylation	Cardioprotective effects of MF might be secondary to eNOS activation via AMPK pathway
Solskov L et al Basic Clinical Pharmacology Toxicology 2008	A: To determine the effects of single dose MF on cardiac protection against IRI S: Wistar rats	Single dose of MF (250mg/kg) vs saline	▪ Reduction of myocardial infarction size ▪ Two fold increased in AMPK-α1 activity	MF might reduce myocardial infarction size in pre-treated subjects via AMPK activation
Saeedi R et al Am J Physiol Heart Cir Physiol 2008	A: Determine if MF has effects on metabolism of heart muscle independent of AMPK pathway S: Sprague-Dawley rats	2mM (this dosage has greatest cellular metabolic effects without an impact on cellular energy status	▪ Increased rate of glycolysis, glucose uptake and fatty acid oxidation ▪ AMPK was not activated by 2mM of MF	MF has AMPK-independent metabolic effects, possibly via protein kinase C and p38 mitogen-activated protein kinase pathways
Kovacic S et al J Biol Chem 2003	A: Akt activation induced by insulin negatively regulates AMPK activities S: Akt transgenic mice and adenovirus infected noenatal rat cardiac myocytes with mutant form of Akt1 and Akt2	5mM of MF	▪ Insulin increased Akt phosphorylation and reduced AMPK phosphorylation ▪ Administration of MF overcome Akt-dependent AMPK suppression ▪ Study suggests a cross talk between Akt and APMK pathway	AMPK can be activated by MF via insulin-independent pathway but higher doses of MF are required
Zhang L et al Am J Physiol Heart Cir Physiol 2007	A: MF activates AMPK in heart via increasing cytosolic AMP S: Sprague-Dawley rats	10mM of MF	▪ MF increases AMPK activity preceded by and correlated with increased cytosolic AMP but overall AMP/ATP remained unchanged	MF activates AMPK without altering total AMP/ATP ratio. High dosage of MF is required for AMPK activation.

(3) THIAZOLIDINEDIONES (TZDS)

Thiazolidinediones (e.g. rosiglitazone and pioglitazone, "TZDs") are ligands for the nuclear hormone receptor family member PPAR-γ (134). Both the TZDs have been shown to activate AMPK in intact cells (307,308). TZDs can also activate AMPK by stimulating the release and expression of circulating adiponectin from adipose tissue (274,275), or indirectly by increasing cellular AMP/ATP ratio, possibly by a similar mechanism to biguanides (309). Both rosiglitazone and pioglitazone have been suggested to have additional and protective beneficial anti-atherosclerotic and anti-inflammatory effects (310). Furthermore, TZDs have also been shown to have diverse beneficial effects on endothelial function, TNF-α, nitric oxide and endothelial cell proliferations via AMPK-dependent and PPARγ-independent mechanisms (311-315). These effects may translate into improvement of clinical outcomes in patients with cardiometabolic disease. Previous studies have raised the intriguing possibilities that these effects may be mediated via AMPK activation (308,316,317). However, like metformin, we are not certain if AMPK activation is the key to these clinical beneficial effects on cardiovascular system. Furthermore, we also need to be very cautious when we try to translate these observations in animal studies to the clinical setting. The doses and type of TZDs that have been shown to activate AMPK vary among different research groups and the doses used in these animal studies may not be applicable to human subjects. Furthermore, the majority of these in vivo studies are short-term studies examining the effects of acute AMPK activation and its metabolic effects. However, the effects of long-term AMP activation by TZDs have yet to be

determined. Nonetheless, the cardiovascular protective effects of TZDs are evidenced in the recently published post hoc analysis from the PROspective pioglitAzone Clinical Trial In macrovascular Events (PROactive) [116]. It showed that patient who has chronic kidney disease who received pioglitazone are less likely to reach composite end-points of all-cause death, MI, and stroke, independent of the severity of renal impairment.

However, it should be noted that TZDs use is associated with the risk of fluid retention, which may exacerbate heart failure (177) (PROACTIVE, United State Food and Drug Administration (FDA) statement). In a recent meta-analysis, Lago and colleagues reported that TZDs increased risk for development of CHF, probably as a result of fluid retention, across a wide background of cardiac risk (relative risk [RR] 1.72, 95% CI 1.21-2.42, p=0.002) (136). There is also a concern that these agents may be associated with additional cardiovascular (MI, stroke) risk in patients with Type 2 diabetes mellitus (T2DM) with rosiglitazone. However, it should be noted that these meta-analyses which included many small trials (138), while large clinical trial data have shown no signal of these CV events (RECORD, PROACTIVE) (177,318). The European Medicines Evaluation Agency (EMEA) for Medicinal Products for Human Use has adopted a scientific opinion in January 2008, recommending the inclusion of a new warning stating that the use of rosiglitazone in patients with ischaemic heart disease and/or peripheral arterial disease is not recommended. A recent FDA review suggested that more large randomized studies with active comparators should be conducted (FDA) by the manufacturers. In 2010, EMEA has recommended the suspension of the

marketing authorisations of rosiglitazone (Avandia, Avandamet) across the European Union.

(4) STATINS

Statins are widely prescribed in patients with metabolic syndrome owing to the high incidence of hypercholesterolaemia in this group of patients. There is mounting evidence to suggest that the clinical benefits of statins are beyond its lipid lowering effects. The clinical efficacy of statins treatment in reducing cardiovascular mortality and morbidity in patients with diabetes and without diabetes are well proven in clinical trials such as the Heart Protection Study and the Collaborative Atorvastatin Diabetes Study (319-322). Besides its cholesterol lowering effect via 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibition, statins has also been shown to activate AMPK in human and bovine endothelial cells (323). Xenos et al have shown that AMPK protein levels in human endothelial cells were increased after being treated with fluvastatin for 2 days (324). Sun et al have also shown that atorvastatin and lovastatin caused rapid activation of AMPK-eNOS/ACC in mouse myocardium and endothelial cells [125]. The atorvastatin dose used in this study was 50mg/kg in mice, which is equivalent to 80mg per day in human. This study has shown that atorvastatin did not alter cellular AMP: ATP ratio, suggesting different mechanism of AMPK activation. The beneficial effects of statins on endothelial function have been suggested to be the results of its ability to up regulate eNOS (319,320,325-327), and its anti-inflammatory and anti-atherogenic effects (322,328).These observations have all suggested that AMPK activation might be the key to the pleiotropic effects of statins on cardiovascular

protection. However, further mechanistic and translational studies are required to show that AMPK activation is indeed the key these effects of statins treatment as well as the examining the doses of different statins required to activate AMPK.

(5) COMPOUND A-769662

Cool et al. have identified a thienopyridone family of AMPK activators (329), compound A-769662. It stimulates AMPK directly in partially purified rat liver and inhibits fatty acid synthesis in primary rat hepatocytes. Short-term treatment of normal Sprague Dawley rats with A-769662 decreases liver malonyl CoA levels and the respiratory exchange ratio, VCO_2/VO_2, indicating an increased rate of whole-body fatty acid oxidation. In ob/ob mice, treatment with compound A-769662 has been shown to decrease plasma glucose, reducing weight gain and significantly decreasing both plasma and liver triglyceride levels. These results demonstrated that small molecule-mediated activation of AMPK in vivo is feasible, and represent therefore a promising approach for the treatment of Type 2 diabetes mellitus and the metabolic syndrome. However, the compound has poor oral bioavailability, limiting its use in clinical settings.

An alternative small molecule compound that is safe, potent, acts directly on AMPK with good oral bioavailability would be an attractive candidate to progress towards clinical development.

CONCLUSIONS

Activation of AMPK pathway may be the key in treating and preventing various cardiometabolic diseases. AMPK pathway and its association with its upstream and downstream kinases have fundamental roles in glucose metabolism, fatty acid oxidation, and protein synthesis. AMPK pathway has been the focus of many researches of late owing to his central role in modulating cardio-metabolic processes, and recent mechanistic studies have shown that AMPK has an important role in the mechanism of action of metformin, thiazolidinediones and statins. Activation of AMPK may be responsible for the insulin sensitizing and beneficial cardio-metabolic effects of these drugs. However, it is still uncertain whether direct activation of the AMPK pathway in the absence of a physiological stress will be beneficial or deleterious overall in humans. It is hoped that chronic activation of AMPK will not result in "over-compensatory" activation of other systems such as the renin-angiotensin-aldosterone system activation in heart failure. Alterations in cardiac AMPK activity are associated with a number of cardiovascular-related diseases such as pathological cardiac hypertrophy (258), myocardial ischemia (244), glycogen storage cardiomyopathy (260), and Wolff-Parkinson-White syndrome (259), suggesting a possible maladaptive role in such conditions. Andersson et al described anti-satiety effects of AMPK, which may lead to weight gain (330). Furthermore, McCullough et al also demonstrated that activated AMPK might be harmful in stroke (331). All these uncertainties will need to be clarified by further translational studies and much effort is still required to define the roles of AMPK activation in various conditions that we have already discussed.

Furthermore, it is also a great challenge for pharmaceutical companies to produce a specific AMPK activator, which has predictable effects owing to its heterotrimeric structure and its complex interactions with various upstream and down stream kinases. The other approach in which many researchers adopted was to develop a compound that targets the down stream kinases of AMPK (i.e. malonyl CoA activator, CPT-1 activator). The AMPK-malonyl CoA-CPT-1 axis might represent an interesting pathway for further research in cardiac substrate utilization and fatty acid metabolism. AMPK-adipocytokines interaction has also formed the rationale of developing of new treatment modalities for the treatment of obesity. Lastly, the AMPK-MTOR-eEF2-p70S6K axis modulations may the key to understand the pathogenesis of cardiac myocytes hypertrophy and mitochondrial biogenesis. The greater understanding of the biochemistry and physiology of AMPK and better understanding of the mechanism of actions of existing agents have nonetheless opened up a new horizon for the treatment and prevention of cardiovascular and metabolic disease.

CHAPTER 8: FINAL DISCUSSION

Diabetes Mellitus and HF commonly coexist, and each condition impact on each other in terms of causation and outcome. DM is highly prevalent amongst HF patients and vice versa. The prevalence of DM increases with severity of HF. Up to one third of patients who are hospitalised with HF are found to be diabetic.

NYHA functional class has been shown to be a predictor of risk of developing DM in HF from population based study, whereas HbA1c measurements are predictors of risk of developing HF in diabetic patients. Elevated HbA1c level is also a predictor of incident HF in diabetics as well as non-diabetics. More importantly, suboptimal glycaemic control as measured by HbA1c is associated with adverse outcome. However, there were some conflicting reports regarding the degree of glycaemic control in T2DM and CHF. In patients with T2DM and CHF, our observational study shows that there is a U shaped relationship between HbA1c and mortality with the lowest mortality risk in patients with modest glycaemic control (HbA1c, $>7 \leq 9\%$). This observational data adds support to the growing concern that we need to redefine the optimal HbA1c level in this high-risk group of patients with co-existing T2DM and CHF. These findings may be partly explained by the differences in severity of CHF, duration of diabetes, and differences in the choices of drugs used.

The bi-directional inter-relationship between CHF and diabetes also extends to insulin resistance (IR). IR precedes and also predicts the development of CHF, independent of established risk factors for CHF including diabetes itself. The degree of IR positively correlates with severity of CHF and is associated with adverse functional consequences (i.e. reduced endothelial function, exercise capacity and associated with abnormal serum biomarkers). The exact pathophysiology of IR and CHF is not fully understood. Activation of SNS, RAS, inflammation, altered adipocytokines levels, formation of advanced glycosylation products, changes in substrate utilization of the myocardium and ED are possible explanations of how IR affecting disease process in CHF. With our increased understanding of the pathophysiological role of IR in CHF, improving IR may represent a new target for treatment for patients with CHF.

So, how can we improve IR? Lifestyle changes such as diet and exercise are possible but very difficult to prescribe, and patients' adherence can be of great challenge. Therefore, pharmacological approach is needed for most patients with evidence of IR/DM and CHF. We are mindful that certain conventional HF medications only have modest beneficial effects on glycaemic control and the prevention of the development of diabetes. Therefore, more potent "insulin sensitizers" are needed to reverse IR in CHF. TZDs were initially thought to be a blockbuster drug when it was first marketed owing to its potent insulin sensitizing property and favourable impacts on cardiovascular parameters such as lipids, blood pressure, inflammatory biomarkers, endothelial function, and fibrinolytic status (332,333). However, its use in CHF was restricted because of its ability to increased fluid retention caused by

increased re-absorption in the distal nephron as well as increased vascular permeability in adipose tissue (334). Furthermore, there are additional concerns regarding the risk of myocardial infarction with TZDs especially with rosiglitazone, which has now been withdrawn from the market.

The incretin system has received a great deal of attention in the treatment of diabetes in recent years. A few randomized controlled trials are currently underway to define the utility of targeting the incretin system in HF patients with DM. Incretin-based therapy may represent a novel therapeutic strategy in the treatment of HF patients with diabetes, as it has been shown to have cardioprotective effects independent of those attributable to tight glycaemic control. Our increased insights and understanding of the incretin system has opened up new horizons in the potential treatment options in CHF, and outcome trials are awaiting.

Metformin, another insulin sensitizing medications, has been on the market for almost 50 years. The use of metformin in patients with CHF has been discouraged previously because of previous experience with phenformin back in the 1970's. The precise mechanisms of action are not fully understood. However, with increased understandings and refreshed insights from accumulating experience of metformin use, metformin has proven to be safe in patients with CHF and DM. More importantly, there are now large observational data and retrospect studies to support the notion that metformin is not only safe, but also its use was associated with a better outcome in patients with CHF and DM. Although these data were encouraging, the main limitations of these observational studies were the potential for selection bias imposed by different

therapies. What is needed is prospective placebo controlled studies to examine the impact of metformin in CHF and DM.

Therefore, in a randomized placebo controlled trial, we evaluatedthe impact of metformin on IR and its effects on exercise capacity in non-diabetic patients with CHF. The primary endpoint of the trial was to determine if improvement of IR with metformin lead to improvement of peak VO_2. However, many patients with CHF are unable to perform maximal exercise and oxygen requirements for activities of daily living rarely approach maximal levels (185). Therefore, we have included the sub-maximal derived exercise variable of the slope of the ratio of minute ventilation to carbon dioxide production (VE/VCO_2) as a secondary end-point of this study. VE/VCO_2 slope is an index of ventilatory response to exercise. Unlike peak VO_2, VE/VCO_2 is not influenced by the mechanical work done during exercise testing but reflects alterations in the peripheries caused by the disease in CHF, which can in turn lead to the progression and symptomatology of CHF (186). Furthermore, we have also explored possible mechanisms of improvement of exercise capacity by measuring left ventricular ejection fraction by echocardiography, endothelial function and related biomarkers.

Our study had two main findings. Firstly, we showed that in CHF patients with insulin resistance, metformin treatment significantly reduced FIRI and this treatment was associated with a weight loss of 1.9 kg. Secondly, although metformin had no effect on peak exercise parameters such as peak VO_2, metformin treatment did result in a significant improvement in VE/VCO_2 slope, a pre-specified endpoint of this proof of concept study. There are several

possible explanations of improved VE/VCO_2 slope. Firstly, the improvement in functional capacity might be related to the weight loss of 1.9 kg associated with metformin therapy. However, in our regression model, we showed that weight reduction alone did not correlate with the reduction in VE/VCO_2 slope. Secondly, the insulin sensitizing properties of metformin might confer some beneficial effects on exercise capacity. Improving insulin sensitivity has been shown to improve exercise capacity. In this regard, we found that with metformin treatment, reduction of FIRI and serum leptin level was significantly correlated with the reduction of VE/VCO_2 slope. Doehner et al had previously demonstrated that hyperleptinaemia is an independent predictor of IR in patients with CHF, and it may play an important role in energy metabolism in these patients (63). Reduction of serum leptin has been previously reported following chronic metformin therapy (206) and might be due to a direct effect of metformin on leptin secretion (207). Recent studies have shown the significance of adipocytokines modulation in HF patients and high levels of adiponectin were associated with adverse outcomes in CHF (75,76). There was no significant change in adiponectin levels in our study ($p=0.168$). However, we did notice that patients in the metformin treated group have decreased adiponectin level, whereas adiponectin levels were higher in the placebo group. Therefore, the improvement of sub-maximal exercise capacity in our cohort may in part be due to an improvement of IR and the reduction of serum leptin level. A third consideration was metformin's ability to activate AMPK, which is expressed in various tissues including the skeletal muscle, myocardium and vascular endothelium (165). Therefore, activation of AMPK could impact on central as well as peripheral haemodynamic mechanisms which in turn leads to

changes in VE/VCO_2 slope and hence, ventilatory class. Improvement of myocardial substrate utilization and glucose uptake through activation of AMPK may improve myocardial contraction and increase cardiac output (196). In animal models of heart failure, metformin has been shown to activate AMPK and improve left ventricular function, and attenuate oxidative stress-induced cardiomyocyte apoptosis, resulting in improved survival (197). However, we did not observe any significant effect of metformin on echo-derived LVEF or CO at rest and during peak exercise. With respect to peripherally mediated mechanisms, effects of metformin on exercising skeletal muscles could account for improvement in VE/VCO_2 slope. Alterations in skeletal muscle energy metabolism, IR and functional adiponectin resistance have been reported and linked to exercise intolerance in patients with CHF (208). A study of the effects of metformin on skeletal muscle enzyme activities in our subjects would be of interest. In our original proposal, we had planned to do this, but this invasive procedure was offered as an option and no patient consented to the procedure. We did not see any significant effect of metformin on endothelial function. Obviously any evidence that metformin improves exercise capacity through central cardiac or peripheral mechanisms in patients with CHF must remain speculative, and cannot be inferred directly from this study. Clearly, further studies are required to define the mechanisms underlying these effects of metformin in CHF.

AMPK pathway has been the focus of recent research, owing to his central role in modulating cardio-metabolic processes. Recent mechanistic studies have shown that AMPK has an important role in the mechanism of

action of metformin, thiazolidinediones and statins. Activation of AMPK may be responsible for the insulin sensitizing and beneficial cardio-metabolic effects of these drugs. For example, in animal models of heart failure, metformin has been shown to activate AMPK, improve left ventricular function and to attenuate oxidative stress-induced cardiomyocyte apoptosis, resulting in improved survival (197). Increased understanding of the beneficial effects of AMPK activation provides the rationale for targeting AMPK in the development of new therapeutic strategies to improve IR, cardio-metabolic disease and heart failure.

PUBLICATIONS AND PRESENTATIONS

PAPERS

1. Aaron K Wong, Allan D Struthers, Anna-Maria Choy, Chim C Lang. Insulin sensitization therapy and the heart: focus on metformin and thiazolidinediones. Heart Fail Clin. 2012 Oct;8(4):539-50. doi: 10.1016/j.hfc.2012.06.002. Epub 2012 Aug 9.

2. Wong AK, Symon R, Alzadjali MA, Ang DS, Ogston S, Choy A, Petrie JR, Struthers AD, Lang CC. The effect of metformin on insulin resistance and exercise parameters in patients with heart failure. Eur J Heart Fail. 2012 Jun 27. [Epub ahead of print]

3. Aaron KF Wong, Jacqueline Howie, John R Petrie, Chim C Lang. *AMP-activated Protein Kinase Pathway: A Potential Therapeutic Target in Cardiometabolic Disease*. Clinical Science 2009. 116(8):607-620. PMID: 19275766

4. Aaron KF Wong, M. ALZadjali, AM Choy, Chim C Lang. *Insulin Resistance: A Potential New Target for Therapy in Patients with Heart Failure*. Cardiovascular Therapeutics, 2008. 26(3):203-13. PMID 18786090

5. Evans JM, Doney AS, AlZadjali MA, Ogston SA, Petrie JR, Morris AD, Struthers AD, Wong AK, Lang CC. *Effect of Metformin on mortality in patients with heart failure and type 2 diabetes mellitus.* Am J Cardiol. 2010 Oct 1;106(7):1006-10. PMID: 20854965

6. MA ALZadjali, V Godgrey, F Khan, AM Choy, AS Doney, AK Wong, JR Petrie, AD Struthers, CC Lang. *Insulin resistance is highly prevalent and is associated with reduced exercise tolerance in non-diabetic patients with heart failure.* Journal of American College of Cardiology. 2009;53:747-753 . PMID 19245964

PRESENTATIONS

1. Title: Metformin in Insulin Resistance LV systolic dysfunction, A Double-blind, placebo controlled trial. (Short-listed for Philip Poole-Wilson Young Investigator award in Clinical Research) in European Society of Cardiology Heart Failure Congress, Berlin May 2010
2. Title: Reversing Insulin Resistance: A New Target for Treatment in Chronic Heart Failure. Annual Meeting of the Association of Physician of Great Britain and Ireland, Dundee April 2010
3. TAYSIDE trial. Scottish Society of Physicians 52nd Annual Meeting, Edinburgh 25th September 2010 (Awarded Fitzgerald Peel Prize)
4. Title: Prevalence of Insulin Resistance and CHF. Scottish Cardiac Society Annual Conference, Glasgow September 2008

POSTERS

1. Moderated poster presentation in European Society of Cardiology Congress, Stockholm, August 2010. *Title: Glycaemic control and the development of heart failure and its importance in diabetic patients with established heart failure*.
2. European Society of Cardiology Congress, Stockholm, August 2010. *Title: Metformin in Insulin Resistance LV systolic dysfunction, A Double-blind, placebo controlled trial.*
3. Moderated poster presentation in British Cardiac Society Annual Scientific Conference, Manchester June 2010. *Title: Metformin in Insulin Resistance LV systolic dysfunction, A Double-blind, placebo controlled trial.*
4. Annual Meeting of the Association of Physician of Great Britain and Ireland, Dundee April 2010. Title: *Metformin in Insulin Resistance LV systolic dysfunction, A Double blind, placebo controlled trial.*
5. Moderated poster presentation in British Cardiac Society Annual Scientific Conference, Manchester June 2008. Title: *Insulin resistance is highly prevalent and is associated with reduced exercise tolerance in non-diabetic patients with heart failure*

REFERENCES

1. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol 1974;34:29-34.
2. Thrainsdottir IS, Aspelund T, Thorgeirsson G et al. The association between glucose abnormalities and heart failure in the population-based Reykjavik study. Diabetes Care 2005;28:612-6.
3. McDonagh TA, Morrison CE, Lawrence A et al. Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population. Lancet 1997;350:829-33.
4. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1979;241:2035-8.
5. MacDonald MR, Petrie MC, Hawkins NM et al. Diabetes, left ventricular systolic dysfunction, and chronic heart failure. Eur Heart J 2008;29:1224-40.
6. Nichols GA, Gullion CM, Koro CE, Ephross SA, Brown JB. The incidence of congestive heart failure in type 2 diabetes: an update. Diabetes Care 2004;27:1879-84.
7. Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405-12.
8. Iribarren C, Karter AJ, Go AS et al. Glycemic control and heart failure among adult patients with diabetes. Circulation 2001;103:2668-73.
9. Amato L, Paolisso G, Cacciatore F et al. Congestive heart failure predicts the development of non-insulin-dependent diabetes mellitus in the elderly. The Osservatorio Geriatrico Regione Campania Group. Diabetes Metab 1997;23:213-8.
10. Tenenbaum A, Motro M, Fisman EZ et al. Functional class in patients with heart failure is associated with the development of diabetes. Am J Med 2003;114:271-5.
11. Shindler D. Diabetes mellitus, a predictor of morbidity and mortality in the Studies of Left Ventricular Dysfunction (SOLVD) Trials and Registry. Am J Cardiol 1996;77.
12. Domanski M, Krause-Steinrauf H, Deedwania P et al. The effect of diabetes on outcomes of patients with advanced heart failure in the BEST trial. J Am Coll Cardiol 2003;42:914-22.
13. Held C, Gerstein HC, Yusuf S et al. Glucose levels predict hospitalization for congestive heart failure in patients at high cardiovascular risk. Circulation 2007;115:1371-5.
14. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:837-53.
15. Ray KK, Seshasai SR, Wijesuriya S et al. Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. Lancet 2009;373:1765-72.

16. Turnbull FM, Abraira C, Anderson RJ et al. Intensive glucose control and macrovascular outcomes in type 2 diabetes. Diabetologia 2009;52:2288-98.
17. Calles-Escandon J, Lovato LC, Simons-Morton DG et al. Effect of intensive compared with standard glycemia treatment strategies on mortality by baseline subgroup characteristics: the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. Diabetes Care 2010;33:721-7.
18. Gerstein HC, Miller ME, Byington RP et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-59.
19. Patel A, MacMahon S, Chalmers J et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560-72.
20. Nesto RW, Bell D, Bonow RO et al. Thiazolidinedione Use, Fluid Retention, and Congestive Heart Failure: A Consensus Statement From the American Heart Association and American Diabetes Association. Circulation 2003;108:2941-2948.
21. Tahrani AA, Varughese GI, Scarpello JH, Hanna FW. Metformin, heart failure, and lactic acidosis: is metformin absolutely contraindicated? BMJ 2007;335:508-12.
22. Pazin-Filho A, Kottgen A, Bertoni AG et al. HbA 1c as a risk factor for heart failure in persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) study. Diabetologia 2008;51:2197-204.
23. Eshaghian S, Horwich TB, Fonarow GC. An unexpected inverse relationship between HbA1c levels and mortality in patients with diabetes and advanced systolic heart failure. Am Heart J 2006;151:91.
24. Gerstein HC, Swedberg K, Carlsson J et al. The hemoglobin A1c level as a progressive risk factor for cardiovascular death, hospitalization for heart failure, or death in patients with chronic heart failure: an analysis of the Candesartan in Heart failure: Assessment of Reduction in Mortality and Morbidity (CHARM) program. Arch Intern Med 2008;168:1699-704.
25. Goode KM, John J, Rigby AS et al. Elevated glycated haemoglobin is a strong predictor of mortality in patients with left ventricular systolic dysfunction who are not receiving treatment for diabetes mellitus. Heart 2009;95:917-23.
26. Aguilar D, Bozkurt B, Ramasubbu K, Deswal A. Relationship of hemoglobin A1C and mortality in heart failure patients with diabetes. J Am Coll Cardiol 2009;54:422-8.
27. Lind M, Olsson M, Rosengren A, Svensson AM, Bounias I, Gudbjornsdottir S. The relationship between glycaemic control and heart failure in 83,021 patients with type 2 diabetes. Diabetologia 2012.
28. Jeffcoate SL. Diabetes control and complications: the role of glycated haemoglobin, 25 years on. Diabet Med 2004;21:657-65.
29. Lind M, Odén A, Fahlén M, Eliasson B. A systematic review of HbA1c variables used in the study of diabetic complications. Diabetes and Metabolic Syndrome: Clinical Research and Reviews 2008;2:282-293.
30. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995;44:968-83.

31. Lind M, Oden A, Fahlen M, Eliasson B. The true value of HbA1c as a predictor of diabetic complications: simulations of HbA1c variables. PLoS One 2009;4:e4412.
32. Nesto RW, Bell D, Bonow RO et al. Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. October 7, 2003. Circulation 2003;108:2941-8.
33. Libby G, MacDonald TM, Evans JM. Record-linkage methodology for prescribing research. J Clin Pharm Ther 2001;26:241-6.
34. Evans JM, Doney AS, AlZadjali MA et al. Effect of Metformin on mortality in patients with heart failure and type 2 diabetes mellitus. Am J Cardiol 2010;106:1006-10.
35. Cox DR. Regression Models and Life-Tables. J Roy Stat Soc B 1972;34:187-&.
36. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. JAMA 2003;290:2159-67.
37. Nathan DM, Cleary PA, Backlund JY et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643-53.
38. Yoshida Y, Hagura R, Hara Y, Sugasawa G, Akanuma Y. Risk factors for the development of diabetic retinopathy in Japanese type 2 diabetic patients. Diabetes Res Clin Pract 2001;51:195-203.
39. Reichard P, Nilsson BY, Rosenqvist U. The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. N Engl J Med 1993;329:304-9.
40. Kosiborod M, Inzucchi SE, Goyal A et al. Relationship between spontaneous and iatrogenic hypoglycemia and mortality in patients hospitalized with acute myocardial infarction. JAMA : the journal of the American Medical Association 2009;301:1556-64.
41. A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. The New England journal of medicine 2001;344:1659-67.
42. Masoudi FA, Inzucchi SE, Wang Y, Havranek EP, Foody JM, Krumholz HM. Thiazolidinediones, metformin, and outcomes in older patients with diabetes and heart failure: an observational study. Circulation 2005;111:583-90.
43. O'Kane MJ, McMenamin M, Bunting BP, Moore A, Coates VE. The relationship between socioeconomic deprivation and metabolic/cardiovascular risk factors in a cohort of patients with type 2 diabetes mellitus. Prim Care Diabetes 2010;4:241-9.
44. Norton GR, Candy G, Woodiwiss AJ. Aminoguanidine Prevents the Decreased Myocardial Compliance Produced by Streptozotocin-Induced Diabetes Mellitus in Rats. Circulation 1996;93:1905-1912.
45. Ceriello A, Esposito K, Ihnat M, Thorpe J, Giugliano D. Effect of acute hyperglycaemia, long-term glycaemic control and insulin on endothelial dysfunction and inflammation in Type 1 diabetic patients with different characteristics. Diabet Med 2010;27:911-7.

46. van Heerebeek L, Hamdani N, Handoko ML et al. Diastolic stiffness of the failing diabetic heart: importance of fibrosis, advanced glycation end products, and myocyte resting tension. Circulation 2008;117:43-51.
47. Bojunga J, Nowak D, Mitrou PS, Hoelzer D, Zeuzem S, Chow KU. Antioxidative treatment prevents activation of death-receptor- and mitochondrion-dependent apoptosis in the hearts of diabetic rats. Diabetologia 2004;47:2072-80.
48. Way KJ, Katai N, King GL. Protein kinase C and the development of diabetic vascular complications. Diabet Med 2001;18:945-59.
49. Ingelsson E, Sundstrom J, Arnlov J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. JAMA 2005;294:334-41.
50. Swan JW, Anker SD, Walton C et al. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. J Am Coll Cardiol 1997;30:527-32.
51. Szlachcic J, Massie BM, Kramer BL, Topic N, Tubau J. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. Am J Cardiol 1985;55:1037-42.
52. Szlachcic J, Massie B, Kramer B, Topic N, J JT. Correlates and prognostic implication of exercise capacity in chronic congestive heart failure. Am J Cardiol 1985; 55:1037-42.
53. Doehner W, Rauchhaus M, Ponikowski P et al. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. J Am Coll Cardiol 2005;46:1019-26.
54. ALZadjali M.A. Khan F. CAM, Struthers A.D., Lang C.C. Insulin Resistance in Heart Failure: Prevalence and Relation to Disease Severity. Journal of cardiac failure 2007;13:S150.
55. AlZadjali MA, Godfrey V, Khan F et al. Insulin resistance is highly prevalent and is associated with reduced exercise tolerance in nondiabetic patients with heart failure. J Am Coll Cardiol 2009;53:747-53.
56. Marshall JD, Bronson RT, Collin GB et al. New Alstrom syndrome phenotypes based on the evaluation of 182 cases. Archives of internal medicine 2005;165:675-83.
57. Nielsen LB, Bartels ED, Bollano E. Overexpression of apolipoprotein B in the heart impedes cardiac triglyceride accumulation and development of cardiac dysfunction in diabetic mice. The Journal of biological chemistry 2002;277:27014-20.
58. Pacher P, Liaudet L, Soriano FG, Mabley JG, Szabo E, Szabo C. The role of poly(ADP-ribose) polymerase activation in the development of myocardial and endothelial dysfunction in diabetes. Diabetes 2002;51:514-21.
59. Trost SU, Belke DD, Bluhm WF, Meyer M, Swanson E, Dillmann WH. Overexpression of the sarcoplasmic reticulum Ca(2+)-ATPase improves myocardial contractility in diabetic cardiomyopathy. Diabetes 2002;51:1166-71.
60. Suarez J, Belke DD, Gloss B et al. In vivo adenoviral transfer of sorcin reverses cardiac contractile abnormalities of diabetic cardiomyopathy. American journal of physiology 2004;286:H68-75.

61. Semeniuk LM, Kryski AJ, Severson DL. Echocardiographic assessment of cardiac function in diabetic db/db and transgenic db/db-hGLUT4 mice. American journal of physiology 2002;283:H976-82.
62. Belke DD, Larsen TS, Gibbs EM, Severson DL. Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice. Am J Physiol Endocrinol Metab 2000;279:E1104-13.
63. Paradise NF, Pilati CF, Payne WR, Finkelstein JA. Left ventricular function of the isolated, genetically obese rat's heart. The American journal of physiology 1985;248:H438-44.
64. Schafer S, Huber J, Wihler C, Rutten H, Busch AE, Linz W. Impaired left ventricular relaxation in type 2 diabetic rats is related to myocardial accumulation of N(epsilon)-(carboxymethyl) lysine. Eur J Heart Fail 2006;8:2-6.
65. Poornima IG, Parikh P, Shannon RP. Diabetic cardiomyopathy: the search for a unifying hypothesis. Circulation research 2006;98:596-605.
66. Nikolaidis LA, Sturzu A, Stolarski C, Elahi D, Shen YT, Shannon RP. The development of myocardial insulin resistance in conscious dogs with advanced dilated cardiomyopathy. Cardiovascular research 2004;61:297-306.
67. Opie LH. The metabolic syndrome - does it exist? A cardiologist's point of view. Cardiovascular journal of Africa 2007;18:S24.
68. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. J Clin Invest 1991;87:2246-52.
69. Gaboury CL, Simonson DC, Seely EW, Hollenberg NK, Williams GH. Relation of pressor responsiveness to angiotensin II and insulin resistance in hypertension. J Clin Invest 1994;94:2295-300.
70. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama 2001;286:327-34.
71. Schmidt MI, Duncan BB, Sharrett AR et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. Lancet 1999;353:1649-52.
72. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation 1997;96:1102-8.
73. Barzilay JI, Abraham L, Heckbert SR et al. The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. Diabetes 2001;50:2384-9.
74. Wisniacki N, Taylor W, Lye M, Wilding JP. Insulin resistance and inflammatory activation in older patients with systolic and diastolic heart failure. Heart 2005;91:32-7.
75. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. The New England journal of medicine 1990;323:236-41.

76. Anker SD, Chua TP, Ponikowski P et al. Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. Circulation 1997;96:526-34.
77. Schulze PC, Kratzsch J, Linke A et al. Elevated serum levels of leptin and soluble leptin receptor in patients with advanced chronic heart failure. Eur J Heart Fail 2003;5:33-40.
78. Furnsinn C, Neschen S, Wagner O, Roden M, Bisschop M, Waldhausl W. Acute and chronic exposure to tumor necrosis factor-alpha fails to affect insulin-stimulated glucose metabolism of isolated rat soleus muscle. Endocrinology 1997;138:2674-9.
79. Chin BS, Blann AD, Gibbs CR, Chung NA, Conway DG, Lip GY. Prognostic value of interleukin-6, plasma viscosity, fibrinogen, von Willebrand factor, tissue factor and vascular endothelial growth factor levels in congestive heart failure. European journal of clinical investigation 2003;33:941-8.
80. Matarese G, La Cava A, Sanna V et al. Balancing susceptibility to infection and autoimmunity: a role for leptin? Trends in immunology 2002;23:182-7.
81. Ahima RS, Flier JS. Leptin. Annual review of physiology 2000;62:413-37.
82. Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR, Grunfeld C. IL-1 beta mediates leptin induction during inflammation. The American journal of physiology 1998;274:R204-8.
83. Considine RV, Premkumar A, Reynolds JC, Sebring NG, Ricks M, Sumner AE. Adiponectin and leptin in African Americans. Obesity (Silver Spring, Md 2008;16:428-34.
84. Lele RD, Joshi SR, Gupte A. Association of adipocytokines (leptin, adiponectin TNF-alpha), insulin and proinsulin with diabetes--the Mumbai Obesity Project [MOP]. The Journal of the Association of Physicians of India 2006;54:689-96.
85. Considine RV, Sinha MK, Heiman ML et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. The New England journal of medicine 1996;334:292-5.
86. Papathanassoglou ED, Moynihan JA, Ackerman MH, Mantzoros CS. Serum leptin levels are higher but are not independently associated with severity or mortality in the multiple organ dysfunction/systemic inflammatory response syndrome: a matched case control and a longitudinal study. Clinical endocrinology 2001;54:225-33.
87. Torpy DJ, Bornstein SR, Chrousos GP. Leptin and interleukin-6 in sepsis. Hormone and metabolic research Hormon- und Stoffwechselforschung 1998;30:726-9.
88. Schols AM, Creutzberg EC, Buurman WA, Campfield LA, Saris WH, Wouters EF. Plasma leptin is related to proinflammatory status and dietary intake in patients with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine 1999;160:1220-6.
89. Minokoshi Y, Kim YB, Peroni OD et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339-43.
90. Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes 1999;48:287-91.

91. Unger RH. Lipotoxic diseases. Annual review of medicine 2002;53:319-36.
92. Toth MJ, Gottlieb SS, Fisher ML, Ryan AS, Nicklas BJ, Poehlman ET. Plasma leptin concentrations and energy expenditure in heart failure patients. Metabolism: clinical and experimental 1997;46:450-3.
93. Leyva F, Anker SD, Egerer K, Stevenson JC, Kox WJ, Coats AJ. Hyperleptinaemia in chronic heart failure. Relationships with insulin. European heart journal 1998;19:1547-51.
94. Doehner W, Rauchhaus M, Godsland IF et al. Insulin resistance in moderate chronic heart failure is related to hyperleptinaemia, but not to norepinephrine or TNF-alpha. Int J Cardiol 2002;83:73-81.
95. Schulze PC, Biolo A, Gopal D et al. Dynamics in insulin resistance and plasma levels of adipokines in patients with acute decompensated and chronic stable heart failure. J Card Fail 2011;17:1004-11.
96. Murdoch DR, Rooney E, Dargie HJ, Shapiro D, Morton JJ, McMurray JJ. Inappropriately low plasma leptin concentration in the cachexia associated with chronic heart failure. Heart (British Cardiac Society) 1999;82:352-6.
97. Filippatos GS, Tsilias K, Venetsanou K et al. Leptin serum levels in cachectic heart failure patients. Relationship with tumor necrosis factor-alpha system. International journal of cardiology 2000;76:117-22.
98. Haluzik M, Parizkova J, Haluzik MM. Adiponectin and its role in the obesity-induced insulin resistance and related complications. Physiol Res 2004;53:123-9.
99. Kumada M, Kihara S, Sumitsuji S et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003;23:85-9.
100. Weyer C, Funahashi T, Tanaka S et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930-5.
101. Kistorp C, Faber J, Galatius S et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation 2005;112:1756-62.
102. George J, Patal S, Wexler D et al. Circulating adiponectin concentrations in patients with congestive heart failure. Heart 2006;92:1420-4.
103. Yin WH, Wei J, Huang WP, Chen JW, Young MS, Lin SJ. Prognostic value of circulating adipokine levels and expressions of adipokines in the myocardium of patients with chronic heart failure. Circ J 2012;76:2139-47.
104. Matsubara M, Namioka K, Katayose S. Decreased plasma adiponectin concentrations in women with low-grade C-reactive protein elevation. Eur J Endocrinol 2003;148:657-62.
105. Ouchi N, Kihara S, Funahashi T et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003;107:671-4.
106. Watanabe S, Tamura T, Ono K et al. Insulin-like growth factor axis (insulin-like growth factor-I/insulin-like growth factor-binding protein-3) as a prognostic predictor of heart failure: association with adiponectin. Eur J Heart Fail 2010;12:1214-22.

107. Van Berendoncks AM, Garnier A, Beckers P et al. Exercise training reverses adiponectin resistance in skeletal muscle of patients with chronic heart failure. Heart 2011;97:1403-9.
108. Steppan CM, Bailey ST, Bhat S et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307-12.
109. Gao CL, Zhao DY, Qiu J et al. Resistin induces rat insulinoma cell RINm5F apoptosis. Molecular biology reports 2009;36:1703-8.
110. Filkova M, Haluzik M, Gay S, Senolt L. The role of resistin as a regulator of inflammation: Implications for various human pathologies. Clin Immunol 2009;133:157-70.
111. Manduteanu I, Dragomir E, Calin M et al. Resistin up-regulates fractalkine expression in human endothelial cells: lack of additive effect with TNF-alpha. Biochem Biophys Res Commun 2009;381:96-101.
112. Pirvulescu M, Manduteanu I, Gan AM et al. A novel pro-inflammatory mechanism of action of resistin in human endothelial cells: up-regulation of SOCS3 expression through STAT3 activation. Biochem Biophys Res Commun 2012;422:321-6.
113. Uslu S, Kebapci N, Kara M, Bal C. Relationship between adipocytokines and cardiovascular risk factors in patients with type 2 diabetes mellitus. Experimental and therapeutic medicine 2012;4:113-120.
114. Osawa H, Ochi M, Kato K et al. Serum resistin is associated with the severity of microangiopathies in type 2 diabetes. Biochem Biophys Res Commun 2007;355:342-6.
115. Zhang MH, Na B, Schiller NB, Whooley MA. Resistin, exercise capacity, and inducible ischemia in patients with stable coronary heart disease: data from the Heart and Soul study. Atherosclerosis 2010;213:604-10.
116. Chemaly ER, Hadri L, Zhang S et al. Long-term in vivo resistin overexpression induces myocardial dysfunction and remodeling in rats. J Mol Cell Cardiol 2011;51:144-55.
117. Frankel DS, Vasan RS, D'Agostino RB, Sr. et al. Resistin, adiponectin, and risk of heart failure the Framingham offspring study. J Am Coll Cardiol 2009;53:754-62.
118. Zhang MH, Na B, Schiller NB, Whooley MA. Association of resistin with heart failure and mortality in patients with stable coronary heart disease: data from the heart and soul study. J Card Fail 2011;17:24-30.
119. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovascular research 2004;63:582-92.
120. Jyothirmayi GN, Soni BJ, Masurekar M, Lyons M, Regan TJ. Effects of Metformin on Collagen Glycation and Diastolic Dysfunction in Diabetic Myocardium. J Cardiovasc Pharmacol Ther 1998;3:319-326.
121. Holmang A, Yoshida N, Jennische E, Waldenstrom A, Bjorntorp P. The effects of hyperinsulinaemia on myocardial mass, blood pressure regulation and central haemodynamics in rats. European journal of clinical investigation 1996;26:973-8.

122. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium, and phosphate in man. J Clin Invest 1975;55:845-55.
123. Nonogaki K. New insights into sympathetic regulation of glucose and fat metabolism. Diabetologia 2000;43:533-49.
124. Sartori M, Ceolotto G, Papparella I et al. Effects of angiotensin II and insulin on ERK1/2 activation in fibroblasts from hypertensive patients. Am J Hypertens 2004;17:604-10.
125. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiological reviews 2005;85:1093-129.
126. Sokos GG, Nikolaidis LA, Mankad S, Elahi D, Shannon RP. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. Journal of cardiac failure 2006;12:694-9.
127. Chiu HC, Kovacs A, Ford DA et al. A novel mouse model of lipotoxic cardiomyopathy. The Journal of clinical investigation 2001;107:813-22.
128. Park TS, Yamashita H, Blaner WS, Goldberg IJ. Lipids in the heart: a source of fuel and a source of toxins. Current opinion in lipidology 2007;18:277-82.
129. Vikramadithyan RK, Hirata K, Yagyu H et al. Peroxisome proliferator-activated receptor agonists modulate heart function in transgenic mice with lipotoxic cardiomyopathy. The Journal of pharmacology and experimental therapeutics 2005;313:586-93.
130. Neubauer S, Horn M, Cramer M et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. Circulation 1997;96:2190-6.
131. Murray AJ, Anderson RE, Watson GC, Radda GK, Clarke K. Uncoupling proteins in human heart. Lancet 2004;364:1786-8.
132. Brand MD, Esteves TC. Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. Cell metabolism 2005;2:85-93.
133. Taegtmeyer H. Switching metabolic genes to build a better heart. Circulation 2002;106:2043-5.
134. Razeghi P, Young ME, Alcorn JL, Moravec CS, Frazier OH, Taegtmeyer H. Metabolic gene expression in fetal and failing human heart. Circulation 2001;104:2923-31.
135. Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990;323:27-36.
136. Ludmer PL, Selwyn AP, Shook TL et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046-51.
137. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37:667-87.
138. Belardinelli R. Endothelial dysfunction in chronic heart failure: clinical implications and therapeutic options. Int J Cardiol 2001;81:1-8.
139. Landmesser U, Hornig B, Drexler H. Endothelial function: a critical determinant in atherosclerosis? Circulation 2004;109:II27-33.

140. Celermajer DS, Sorensen KE, Gooch VM et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111-5.
141. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000;87:840-4.
142. Hogikyan RV, Galecki AT, Pitt B, Halter JB, Greene DA, Supiano MA. Specific impairment of endothelium-dependent vasodilation in subjects with type 2 diabetes independent of obesity. J Clin Endocrinol Metab 1998;83:1946-52.
143. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 1996;97:2601-10.
144. Anastasiou E, Lekakis JP, Alevizaki M et al. Impaired endothelium-dependent vasodilatation in women with previous gestational diabetes. Diabetes Care 1998;21:2111-5.
145. Prasad A, Higano ST, Al Suwaidi J et al. Abnormal coronary microvascular endothelial function in humans with asymptomatic left ventricular dysfunction. Am Heart J 2003;146:549-54.
146. Treasure CB, Vita JA, Cox DA et al. Endothelium-dependent dilation of the coronary microvasculature is impaired in dilated cardiomyopathy. Circulation 1990;81:772-9.
147. Cersosimo E, DeFronzo RA. Insulin resistance and endothelial dysfunction: the road map to cardiovascular diseases. Diabetes/metabolism research and reviews 2006;22:423-36.
148. Hamburg NM, Larson MG, Vita JA et al. Metabolic syndrome, insulin resistance, and brachial artery vasodilator function in Framingham Offspring participants without clinical evidence of cardiovascular disease. Am J Cardiol 2008;101:82-8.
149. Ardigo D, Franzini L, Valtuena S, Monti LD, Reaven GM, Zavaroni I. Relation of plasma insulin levels to forearm flow-mediated dilatation in healthy volunteers. Am J Cardiol 2006;97:1250-4.
150. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888-904.
151. Sjoholm A, Nystrom T. Endothelial inflammation in insulin resistance. Lancet 2005;365:610-2.
152. Lundman P, Eriksson MJ, Stuhlinger M, Cooke JP, Hamsten A, Tornvall P. Mild-to-moderate hypertriglyceridemia in young men is associated with endothelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine. J Am Coll Cardiol 2001;38:111-6.
153. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000;101:948-54.
154. Knowler WC, Barrett-Connor E, Fowler SE et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403.

155. Andraws R, Brown DL. Effect of inhibition of the renin-angiotensin system on development of type 2 diabetes mellitus (meta-analysis of randomized trials). Am J Cardiol 2007;99:1006-12.
156. Fonseca V, Bakris GL, Bell DS et al. Differential effect of beta-blocker therapy on insulin resistance as a function of insulin sensitizer use: results from GEMINI. Diabet Med 2007;24:759-63.
157. Torp-Pedersen C, Metra M, Charlesworth A et al. Effects of metoprolol and carvedilol on pre-existing and new onset diabetes in patients with chronic heart failure: data from the Carvedilol Or Metoprolol European Trial (COMET). Heart 2007;93:968-73.
158. Martinez FA. Aldosterone inhibition and cardiovascular protection: more important than it once appeared. Cardiovasc Drugs Ther 2010;24:345-50.
159. Pitt B, Zannad F, Remme WJ et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999;341:709-17.
160. Pitt B, Williams G, Remme W et al. The EPHESUS trial: eplerenone in patients with heart failure due to systolic dysfunction complicating acute myocardial infarction. Eplerenone Post-AMI Heart Failure Efficacy and Survival Study. Cardiovasc Drugs Ther 2001;15:79-87.
161. Swedberg K, Zannad F, McMurray JJ et al. Eplerenone and atrial fibrillation in mild systolic heart failure: results from the EMPHASIS-HF (Eplerenone in Mild Patients Hospitalization And SurvIval Study in Heart Failure) study. J Am Coll Cardiol 2012;59:1598-603.
162. Homma T, Fujisawa M, Arai K, Ishii M, Sada T, Ikeda M. Spironolactone, but not Eplerenone, Impairs Glucose Tolerance in a Rat Model of Metabolic Syndrome. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 2012;74:1015-22.
163. Yamaji M, Tsutamoto T, Kawahara C et al. Effect of eplerenone versus spironolactone on cortisol and hemoglobin A(c) levels in patients with chronic heart failure. Am Heart J 2010;160:915-21.
164. Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). The Journal of biological chemistry 1995;270:12953-6.
165. Nissen SE, Nicholls SJ, Wolski K et al. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. Jama 2008;299:1561-73.
166. Lago RM, Singh PP, Nesto RW. Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. Lancet 2007;370:1129-36.
167. Singh S, Loke YK, Furberg CD. Long-term risk of cardiovascular events with rosiglitazone: a meta-analysis. JAMA 2007;298:1189-95.
168. Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 2007;356:2457-71.

169. Ungar G, Freedman L, Shapiro SL. Pharmacological studies of a new oral hypoglycemic drug. Proc Soc Exp Biol Med 1957;95:190-2.
170. Bailey CJ, Turner RC. Metformin. N Engl J Med 1996;334:574-9.
171. lucophage & Glucophage XR prescribing Information. Bristol-Myers Squibb Company

, 2003.
172. Misbin R. The phantom of lactic acidosis due to metformin in patients with diabetes. Diabetes Care 2002;25:2244-2248.
173. Salpeter SR, Greyber E, Pasternak GA, Salpeter EE. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus: systematic review and meta-analysis. Archives of internal medicine 2003;163:2594-602.
174. Lalau J, Race J. Lactic acidosis in metformin-treated patients: prognostic value of arterial lactate levels and plasma metformin concentrations. Drug Saf 1999;20:377-384.
175. Stades A, Heikens J, Erkeleens D, Holleman F, Hoekstra J. Metformin and lactic acidosis: cause or coincidence? A review of case reports. J Intern Med 2004;255:179-187.
176. Dargie HJ, Hildebrandt PR, Riegger GA et al. A randomized, placebo-controlled trial assessing the effects of rosiglitazone on echocardiographic function and cardiac status in type 2 diabetic patients with New York Heart Association Functional Class I or II Heart Failure. J Am Coll Cardiol 2007;49:1696-704.
177. Elrick H, Stimmler L, Hlad CJ, Jr., Arai Y. Plasma Insulin Response to Oral and Intravenous Glucose Administration. The Journal of clinical endocrinology and metabolism 1964;24:1076-82.
178. Drucker DJ. The biology of incretin hormones. Cell metabolism 2006;3:153-65.
179. Farilla L, Bulotta A, Hirshberg B et al. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. Endocrinology 2003;144:5149-58.
180. Farilla L, Hui H, Bertolotto C et al. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology 2002;143:4397-408.
181. Nikolaidis LA, Elahi D, Hentosz T et al. Recombinant glucagon-like peptide-1 increases myocardial glucose uptake and improves left ventricular performance in conscious dogs with pacing-induced dilated cardiomyopathy. Circulation 2004;110:955-61.
182. Vila Petroff MG, Egan JM, Wang X, Sollott SJ. Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. Circulation research 2001;89:445-52.
183. Nystrom T, Gutniak MK, Zhang Q et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. American journal of physiology 2004;287:E1209-15.
184. Barragan JM, Rodriguez RE, Blazquez E. Changes in arterial blood pressure and heart rate induced by glucagon-like peptide-1-(7-36) amide in rats. The American journal of physiology 1994;266:E459-66.

185. Edwards CM, Edwards AV, Bloom SR. Cardiovascular and pancreatic endocrine responses to glucagon-like peptide-1(7-36) amide in the conscious calf. Experimental physiology 1997;82:709-16.
186. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet 2006;368:1696-705.
187. Yin M, Sillje HH, Meissner M, van Gilst WH, de Boer RA. Early and late effects of the DPP-4 inhibitor vildagliptin in a rat model of post-myocardial infarction heart failure. Cardiovasc Diabetol 2011;10:85.
188. Chaykovska L, von Websky K, Rahnenfuhrer J et al. Effects of DPP-4 inhibitors on the heart in a rat model of uremic cardiomyopathy. PLoS One 2011;6:e27861.
189. Gomez N, Touihri K, Matheeussen V et al. Dipeptidyl peptidase IV inhibition improves cardiorenal function in overpacing-induced heart failure. Eur J Heart Fail 2012;14:14-21.
190. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families. The Framingham offspring study. Am J Epidemiol 1979;110:281-90.
191. Stafylas PC, Sarafidis PA, Lasaridis AN. The controversial effects of thiazolidinediones on cardiovascular morbidity and mortality. Int J Cardiol 2009;131:298-304.
192. Furuse Y, Ogino K, Shimoyama M, Sasaki N, Hisatome I. Ca(2+)-sensitizing effect is involved in the positive inotropic effect of troglitazone. British journal of pharmacology 2001;133:1307-13.
193. Wong AK, AlZadjali MA, Choy AM, Lang CC. Insulin resistance: a potential new target for therapy in patients with heart failure. Cardiovasc Ther 2008;26:203-13.
194. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with sulfonylurea monotherapy in type 2 diabetes. Diabetes Care 2002;25:2244-8.
195. Tsuji T, Mizushige K, Noma T et al. Pioglitazone improves left ventricular diastolic function and decreases collagen accumulation in prediabetic stage of a type II diabetic rat. J Cardiovasc Pharmacol 2001;38:868-74.
196. Asakawa M, Takano H, Nagai T et al. Peroxisome proliferator-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. Circulation 2002;105:1240-6.
197. Evans JM, Ogston SA, Emslie-Smith A, Morris AD. Risk of mortality and adverse cardiovascular outcomes in type 2 diabetes: a comparison of patients treated with sulfonylureas and metformin. Diabetologia 2006;49:930-6.
198. ALZadjali. Metformin use in associated with markedly lower clinical outcomes in patients with heart failure and Type 2 diabetes. Clinical Pharmacology & Therapeutics 2008;83:s5.
199. Morris AD, Boyle DI, MacAlpine R et al. The diabetes audit and research in Tayside Scotland (DARTS) study: electronic record linkage to create a diabetes register. DARTS/MEMO Collaboration. BMJ 1997;315:524-8.

200. Karalliedde J, Buckingham R, Starkie M, Lorand D, Stewart M, Viberti G. Effect of various diuretic treatments on rosiglitazone-induced fluid retention. Journal of the American Society of Nephrology : JASN 2006;17:3482-90.
201. Coats AJ, Anker SD. Insulin resistance in chronic heart failure. J Cardiovasc Pharmacol 2000;35:S9-14.
202. Suskin N, McKelvie RS, Burns RJ et al. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. Eur Heart J 2000;21:1368-75.
203. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. Ann Intern Med 2002;137:25-33.
204. Dormandy JA, Charbonnel B, Eckland DJ et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. Lancet 2005;366:1279-89.
205. Tzoulaki I, Molokhia M, Curcin V et al. Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. BMJ 2009;339:b4731.
206. Asensio-Lopez MC, Lax A, Pascual-Figal DA, Valdes M, Sanchez-Mas J. Metformin protects against doxorubicin-induced cardiotoxicity: involvement of the adiponectin cardiac system. Free Radic Biol Med 2011;51:1861-71.
207. Russell RR, 3rd, Li J, Coven DL et al. AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. J Clin Invest 2004;114:495-503.
208. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. J Am Coll Cardiol 2001;37:1344-50.
209. Egstrup M, Schou M, Gustafsson I, Kistorp CN, Hildebrandt PR, Tuxen CD. Oral glucose tolerance testing in an outpatient heart failure clinic reveals a high proportion of undiagnosed diabetic patients with an adverse prognosis. Eur J Heart Fail 2010;13:319-26.
210. Salpeter S, Greyber E, Pasternak G, Salpeter E. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev 2006:CD002967.
211. Eurich DT, McAlister FA, Blackburn DF et al. Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: systematic review. BMJ 2007;335:497.
212. Goda A, Lang CC, Williams P, Jones M, Farr MJ, Mancini DM. Usefulness of non-invasive measurement of cardiac output during sub-maximal exercise to predict outcome in patients with chronic heart failure. Am J Cardiol 2009;104:1556-60.
213. Lang CC, Agostoni P, Mancini DM. Prognostic significance and measurement of exercise-derived hemodynamic variables in patients with heart failure. J Card Fail 2007;13:672-9.
214. Caballero AE, Delgado A, Aguilar-Salinas CA et al. The differential effects of metformin on markers of endothelial activation and inflammation in

subjects with impaired glucose tolerance: a placebo-controlled, randomized clinical trial. J Clin Endocrinol Metab 2004;89:3943-8.

215. Lang CC, Karlin P, Haythe J, Tsao L, Mancini DM. Ease of noninvasive measurement of cardiac output coupled with peak VO2 determination at rest and during exercise in patients with heart failure. Am J Cardiol 2007;99:404-5.
216. Corretti MC, Anderson TJ, Benjamin EJ et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257-65.
217. Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol 2003;42:1149-60.
218. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension 2011;57:363-9.
219. Anderson TJ, Uehata A, Gerhard MD et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 1995;26:1235-41.
220. Hamburg NM, Benjamin EJ. Assessment of endothelial function using digital pulse amplitude tonometry. Trends in cardiovascular medicine 2009;19:6-11.
221. Paolisso G, De Riu S, Marrazzo G, Verza M, Varricchio M, D'Onofrio F. Insulin resistance and hyperinsulinemia in patients with chronic congestive heart failure. Metabolism 1991;40:972-7.
222. Witteles RM, Fowler MB. Insulin-resistant cardiomyopathy clinical evidence, mechanisms, and treatment options. J Am Coll Cardiol 2008;51:93-102.
223. Gundewar S, Calvert JW, Jha S et al. Activation of AMP-activated protein kinase by metformin improves left ventricular function and survival in heart failure. Circ Res 2009;104:403-11.
224. Sasaki H, Asanuma H, Fujita M et al. Metformin prevents progression of heart failure in dogs: role of AMP-activated protein kinase. Circulation 2009;119:2568-77.
225. Zou MH, Kirkpatrick SS, Davis BJ et al. Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. J Biol Chem 2004;279:43940-51.
226. Cuthbertson J, Patterson S, O'Harte FP, Bell PM. Investigation of the effect of oral metformin on dipeptidylpeptidase-4 (DPP-4) activity in Type 2 diabetes. Diabet Med 2009;26:649-54.
227. Maida A, Lamont BJ, Cao X, Drucker DJ. Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-alpha in mice. Diabetologia 2010;54:339-49.
228. Gitt AK, Wasserman K, Kilkowski C et al. Exercise anaerobic threshold and ventilatory efficiency identify heart failure patients for high risk of early death. Circulation 2002;106:3079-84.
229. Ingle L, Sloan R, Carroll S, Goode K, Cleland JG, Clark AL. Prognostic significance of different measures of the ventilation-carbon dioxide relation in patients with suspected heart failure. Eur J Heart Fail 2011;13:537-42.

230. Arena R, Myers J, Abella J et al. Development of a ventilatory classification system in patients with heart failure. Circulation 2007;115:2410-7.
231. Beck-da-Silva L, Higginson L, Fraser M, Williams K, Haddad H. Effect of Orlistat in obese patients with heart failure: a pilot study. Congest Heart Fail 2005;11:118-23.
232. Regensteiner JG, Bauer TA, Reusch JE. Rosiglitazone improves exercise capacity in individuals with type 2 diabetes. Diabetes Care 2005;28:2877-83.
233. Glueck CJ, Fontaine RN, Wang P et al. Metformin reduces weight, centripetal obesity, insulin, leptin, and low-density lipoprotein cholesterol in nondiabetic, morbidly obese subjects with body mass index greater than 30. Metabolism 2001;50:856-61.
234. Klein J, Westphal S, Kraus D et al. Metformin inhibits leptin secretion via a mitogen-activated protein kinase signalling pathway in brown adipocytes. J Endocrinol 2004;183:299-307.
235. Ventura-Clapier R, Garnier A, Veksler V. Energy metabolism in heart failure. J Physiol 2004;555:1-13.
236. Nieuwenhuis-Ruifrok AE, Kuchenbecker WK, Hoek A, Middleton P, Norman RJ. Insulin sensitizing drugs for weight loss in women of reproductive age who are overweight or obese: systematic review and meta-analysis. Hum Reprod Update 2009;15:57-68.
237. Ford ES. Prevalence of the metabolic syndrome defined by the International Diabetes Federation among adults in the U.S. Diabetes care 2005;28:2745-9.
238. Ford ES. Risks for all-cause mortality, cardiovascular disease, and diabetes associated with the metabolic syndrome: a summary of the evidence. Diabetes care 2005;28:1769-78.
239. Ford ES. Prevalence of the metabolic syndrome in US populations. Endocrinology and metabolism clinics of North America 2004;33:333-50.
240. Bergeron R, Russell RR, 3rd, Young LH et al. Effect of AMPK activation on muscle glucose metabolism in conscious rats. The American journal of physiology 1999;276:E938-44.
241. Russell RR, 3rd, Bergeron R, Shulman GI, Young LH. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. Am J Physiol 1999;277:H643-9.
242. Ruderman NB, Cacicedo JM, Itani S et al. Malonyl-CoA and AMP-activated protein kinase (AMPK): possible links between insulin resistance in muscle and early endothelial cell damage in diabetes. Biochemical Society transactions 2003;31:202-6.
243. Long YC, Barnes BR, Mahlapuu M et al. Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. Diabetologia 2005;48:2354-64.
244. Berasi SP, Huard C, Li D et al. Inhibition of gluconeogenesis through transcriptional activation of EGR1 and DUSP4 by AMP-activated kinase. J Biol Chem 2006;281:27167-77.
245. Carlson CA, Kim KH. Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. The Journal of biological chemistry 1973;248:378-80.

246. Ingebritsen TS, Lee HS, Parker RA, Gibson DM. Reversible modulation of the activities of both liver microsomal hydroxymethylglutaryl coenzyme A reductase and its inactivating enzyme. Evidence for regulation by phosphorylation-dephosphorylation. Biochemical and biophysical research communications 1978;81:1268-77.
247. Carling D, Zammit VA, Hardie DG. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. FEBS letters 1987;223:217-22.
248. Munday MR, Campbell DG, Carling D, Hardie DG. Identification by amino acid sequencing of three major regulatory phosphorylation sites on rat acetyl-CoA carboxylase. European journal of biochemistry / FEBS 1988;175:331-8.
249. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. Endocrinology 2003;144:5179-83.
250. Polekhina G, Gupta A, Michell BJ et al. AMPK beta subunit targets metabolic stress sensing to glycogen. Curr Biol 2003;13:867-71.
251. Bateman A. The structure of a domain common to archaebacteria and the homocystinuria disease protein. Trends in biochemical sciences 1997;22:12-3.
252. Scott JW, Hawley SA, Green KA et al. CBS domains form energy-sensing modules whose binding of adenosine ligands is disrupted by disease mutations. The Journal of clinical investigation 2004;113:274-84.
253. Woods A, Johnstone SR, Dickerson K et al. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr Biol 2003;13:2004-8.
254. Hawley SA, Boudeau J, Reid JL et al. Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. Journal of biology 2003;2:28.
255. Shaw RJ, Kosmatka M, Bardeesy N et al. The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Proceedings of the National Academy of Sciences of the United States of America 2004;101:3329-35.
256. Lizcano JM, Goransson O, Toth R et al. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. The EMBO journal 2004;23:833-43.
257. Jaleel M, McBride A, Lizcano JM et al. Identification of the sucrose non-fermenting related kinase SNRK, as a novel LKB1 substrate. FEBS letters 2005;579:1417-23.
258. Hemminki A, Markie D, Tomlinson I et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391:184-7.
259. Buhl ES, Jessen N, Pold R et al. Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. Diabetes 2002;51:2199-206.
260. Luiken JJ, Coort SL, Willems J et al. Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling. Diabetes 2003;52:1627-34.

261. Marsin AS, Bertrand L, Rider MH et al. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. Curr Biol 2000;10:1247-55.
262. Krause U, Bertrand L, Hue L. Control of p70 ribosomal protein S6 kinase and acetyl-CoA carboxylase by AMP-activated protein kinase and protein phosphatases in isolated hepatocytes. European journal of biochemistry / FEBS 2002;269:3751-9.
263. Horman S, Browne G, Krause U et al. Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. Curr Biol 2002;12:1419-23.
264. Young LH, Renfu Y, Russell R et al. Low-flow ischemia leads to translocation of canine heart GLUT-4 and GLUT-1 glucose transporters to the sarcolemma in vivo. Circulation 1997;95:415-22.
265. Shibata R, Sato K, Pimentel DR et al. Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. Nat Med 2005;11:1096-103.
266. Li J, Coven DL, Miller EJ et al. Activation of AMPK alpha- and gamma-isoform complexes in the intact ischemic rat heart. American journal of physiology 2006;291:H1927-34.
267. Xing Y, Musi N, Fujii N et al. Glucose metabolism and energy homeostasis in mouse hearts overexpressing dominant negative alpha2 subunit of AMP-activated protein kinase. The Journal of biological chemistry 2003;278:28372-7.
268. Calvert JW, Gundewar S, Jha S et al. Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling. Diabetes 2008;57:696-705.
269. Sakamoto K, Zarrinpashneh E, Budas GR et al. Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1. Am J Physiol Endocrinol Metab 2006;290:E780-8.
270. Baron SJ, Li J, Russell RR, 3rd et al. Dual mechanisms regulating AMPK kinase action in the ischemic heart. Circ Res 2005;96:337-45.
271. Dyck JR, Lopaschuk GD. AMPK alterations in cardiac physiology and pathology: enemy or ally? J Physiol 2006;574:95-112.
272. Lopaschuk GD, Collins-Nakai R, Olley PM et al. Plasma fatty acid levels in infants and adults after myocardial ischemia. American heart journal 1994;128:61-7.
273. Hendrickson SC, St Louis JD, Lowe JE, Abdel-aleem S. Free fatty acid metabolism during myocardial ischemia and reperfusion. Molecular and cellular biochemistry 1997;166:85-94.
274. Lopaschuk GD, Spafford MA. Energy substrate utilization by isolated working hearts from newborn rabbits. The American journal of physiology 1990;258:H1274-80.
275. Gollob MH, Seger JJ, Gollob TN et al. Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy. Circulation 2001;104:3030-3.

276. Davies JK, Wells DJ, Liu K et al. Characterization of the role of gamma2 R531G mutation in AMP-activated protein kinase in cardiac hypertrophy and Wolff-Parkinson-White syndrome. American journal of physiology 2006;290:H1942-51.
277. Sidhu JS, Rajawat YS, Rami TG et al. Transgenic mouse model of ventricular preexcitation and atrioventricular reentrant tachycardia induced by an AMP-activated protein kinase loss-of-function mutation responsible for Wolff-Parkinson-White syndrome. Circulation 2005;111:21-9.
278. Carling D, Woods A, Thornton C et al. Molecular characterization of the AMP-activated protein kinase and its role in cellular metabolism. Biochemical Society transactions 1997;25:1224-8.
279. Daniel T, Carling D. Functional analysis of mutations in the gamma 2 subunit of AMP-activated protein kinase associated with cardiac hypertrophy and Wolff-Parkinson-White syndrome. The Journal of biological chemistry 2002;277:51017-24.
280. Murphy RT, Mogensen J, McGarry K et al. Adenosine monophosphate-activated protein kinase disease mimicks hypertrophic cardiomyopathy and Wolff-Parkinson-White syndrome: natural history. Journal of the American College of Cardiology 2005;45:922-30.
281. Tian R, Musi N, D'Agostino J, Hirshman MF, Goodyear LJ. Increased adenosine monophosphate-activated protein kinase activity in rat hearts with pressure-overload hypertrophy. Circulation 2001;104:1664-9.
282. Bolster DR, Crozier SJ, Kimball SR, Jefferson LS. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. J Biol Chem 2002;277:23977-80.
283. Chan AY, Soltys CL, Young ME, Proud CG, Dyck JR. Activation of AMP-activated protein kinase inhibits protein synthesis associated with hypertrophy in the cardiac myocyte. J Biol Chem 2004;279:32771-9.
284. Arad M, Benson DW, Perez-Atayde AR et al. Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. The Journal of clinical investigation 2002;109:357-62.
285. Blair E, Redwood C, Ashrafian H et al. Mutations in the gamma(2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: evidence for the central role of energy compromise in disease pathogenesis. Human molecular genetics 2001;10:1215-20.
286. Gollob MH, Green MS, Tang AS et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. The New England journal of medicine 2001;344:1823-31.
287. Arad M, Moskowitz IP, Patel VV et al. Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff-Parkinson-White syndrome in glycogen storage cardiomyopathy. Circulation 2003;107:2850-6.
288. Shibata R, Ouchi N, Ito M et al. Adiponectin-mediated modulation of hypertrophic signals in the heart. Nature medicine 2004;10:1384-9.
289. Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. The Journal of biological chemistry 2003;278:45021-6.

290. Chen ZP, Mitchelhill KI, Michell BJ et al. AMP-activated protein kinase phosphorylation of endothelial NO synthase. FEBS letters 1999;443:285-9.
291. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological reviews 1991;43:109-42.
292. Suzuki K, Uchida K, Nakanishi N, Hattori Y. Cilostazol activates AMP-activated protein kinase and restores endothelial function in diabetes. Am J Hypertens 2008;21:451-7.
293. Morrow VA, Foufelle F, Connell JM, Petrie JR, Gould GW, Salt IP. Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells. The Journal of biological chemistry 2003;278:31629-39.
294. Xie Z, Dong Y, Scholz R, Neumann D, Zou MH. Phosphorylation of LKB1 at serine 428 by protein kinase C-zeta is required for metformin-enhanced activation of the AMP-activated protein kinase in endothelial cells. Circulation 2008;117:952-62.
295. Boyle JG LP, Ewart MA, Reihill JA, Ritchie SA, Connell JM, Cleland SJ, Salt IP. Rosiglitazone stimulates nitric oxide synthesis in human aortic endothelial cells via AMP-activated protein kinase. J Biol Chem 2008;283:11210-7.
296. Lee M, Hwang JT, Lee HJ et al. AMP-activated protein kinase activity is critical for hypoxia-inducible factor-1 transcriptional activity and its target gene expression under hypoxic conditions in DU145 cells. The Journal of biological chemistry 2003;278:39653-61.
297. Reihill JA, Ewart MA, Hardie DG, Salt IP. AMP-activated protein kinase mediates VEGF-stimulated endothelial NO production. Biochemical and biophysical research communications 2007;354:1084-8.
298. Nagata D, Takeda R, Sata M et al. AMP-activated protein kinase inhibits angiotensin II-stimulated vascular smooth muscle cell proliferation. Circulation 2004;110:444-51.
299. Kim JE, Kim YW, Lee IK, Kim JY, Kang YJ, Park SY. AMP-activated protein kinase activation by 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) inhibits palmitate-induced endothelial cell apoptosis through reactive oxygen species suppression. J Pharmacol Sci 2008;106:394-403.
300. Ingelsson E, Larson MG, Yin X et al. Circulating Ghrelin, Leptin, and Soluble Leptin Receptor concentrations and Cardiometabolic Risk Factors in a Community-Based Sample. The Journal of clinical endocrinology and metabolism 2008.
301. Yamauchi T, Kamon J, Minokoshi Y et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nature medicine 2002;8:1288-95.
302. Tomas E, Tsao TS, Saha AK et al. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proceedings of the National Academy of Sciences of the United States of America 2002;99:16309-13.
303. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763-70.

304. Viollet B, Foretz M, Guigas B et al. Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. The Journal of physiology 2006;574:41-53.
305. Minokoshi Y, Alquier T, Furukawa N et al. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 2004;428:569-74.
306. Gonon AT, Widegren U, Bulhak A et al. Adiponectin protects against myocardial ischaemia-reperfusion injury via AMP-activated protein kinase, Akt, and nitric oxide. Cardiovascular research 2008;78:116-22.
307. Liao Y, Takashima S, Maeda N et al. Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. Cardiovascular research 2005;67:705-13.
308. Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. The American journal of physiology 1997;273:E1107-12.
309. Mangano DT. Effects of acadesine on myocardial infarction, stroke, and death following surgery. A meta-analysis of the 5 international randomized trials. The Multicenter Study of Perioperative Ischemia (McSPI) Research Group. Jama 1997;277:325-32.
310. Song XM, Fiedler M, Galuska D et al. 5-Aminoimidazole-4-carboxamide ribonucleoside treatment improves glucose homeostasis in insulin-resistant diabetic (ob/ob) mice. Diabetologia 2002;45:56-65.
311. Bergeron R, Previs SF, Cline GW et al. Effect of 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside infusion on in vivo glucose and lipid metabolism in lean and obese Zucker rats. Diabetes 2001;50:1076-82.
312. Iglesias MA, Ye JM, Frangioudakis G et al. AICAR administration causes an apparent enhancement of muscle and liver insulin action in insulin-resistant high-fat-fed rats. Diabetes 2002;51:2886-94.
313. Cuthbertson DJ, Babraj JA, Mustard KJ et al. 5-aminoimidazole-4-carboxamide 1-beta-D-ribofuranoside acutely stimulates skeletal muscle 2-deoxyglucose uptake in healthy men. Diabetes 2007;56:2078-84.
314. Lihn AS, Jessen N, Pedersen SB, Lund S, Richelsen B. AICAR stimulates adiponectin and inhibits cytokines in adipose tissue. Biochemical and biophysical research communications 2004;316:853-8.
315. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. American journal of physiology 2001;280:E745-51.
316. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. Diabetes 2002;51:3391-9.
317. Hotamisligil GS. The role of TNFalpha and TNF receptors in obesity and insulin resistance. Journal of internal medicine 1999;245:621-5.
318. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 1998;352:854-65.
319. Zhang L, He H, Balschi JA. Metformin and phenformin activate AMP-activated protein kinase in the heart by increasing cytosolic AMP concentration. American journal of physiology 2007;293:H457-66.

320. Yang J, Holman GD. Long-term metformin treatment stimulates cardiomyocyte glucose transport through an AMP-activated protein kinase-dependent reduction in GLUT4 endocytosis. Endocrinology 2006;147:2728-36.
321. Kovacic S, Soltys CL, Barr AJ, Shiojima I, Walsh K, Dyck JR. Akt activity negatively regulates phosphorylation of AMP-activated protein kinase in the heart. The Journal of biological chemistry 2003;278:39422-7.
322. Zhou G, Myers R, Li Y et al. Role of AMP-activated protein kinase in mechanism of metformin action. The Journal of clinical investigation 2001;108:1167-74.
323. Musi N, Hirshman MF, Nygren J et al. Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes 2002;51:2074-81.
324. Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. Diabetes 2006;55:496-505.
325. McCarty MF. AMPK activation as a strategy for reversing the endothelial lipotoxicity underlying the increased vascular risk associated with insulin resistance syndrome. Medical hypotheses 2005;64:1211-5.
326. Buzzai M, Jones RG, Amaravadi RK et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer research 2007;67:6745-52.
327. Hawley SA, Gadalla AE, Olsen GS, Hardie DG. The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. Diabetes 2002;51:2420-5.
328. El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. The Journal of biological chemistry 2000;275:223-8.
329. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J 2000;348 Pt 3:607-14.
330. Jonker JW, Schinkel AH. Pharmacological and physiological functions of the polyspecific organic cation transporters: OCT1, 2, and 3 (SLC22A1-3). The Journal of pharmacology and experimental therapeutics 2004;308:2-9.
331. Bergheim I, Guo L, Davis MA et al. Metformin prevents alcohol-induced liver injury in the mouse: Critical role of plasminogen activator inhibitor-1. Gastroenterology 2006;130:2099-112.
332. Saeedi R, Parsons HL, Wambolt RB et al. Metabolic actions of metformin in the heart can occur by AMPK-independent mechanisms. American journal of physiology 2008;294:H2497-506.
333. Bertrand L, Ginion A, Beauloye C et al. AMPK activation restores the stimulation of glucose uptake in an in vitro model of insulin-resistant cardiomyocytes via the activation of protein kinase B. Am J Physiol Heart Circ Physiol 2006;291:H239-50.

334. Saha AK, Avilucea PR, Ye JM, Assifi MM, Kraegen EW, Ruderman NB. Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue in vivo. Biochemical and biophysical research communications 2004;314:580-5.
335. Fryer LG, Parbu-Patel A, Carling D. The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. The Journal of biological chemistry 2002;277:25226-32.
336. Brunmair B, Staniek K, Gras F et al. Thiazolidinediones, like metformin, inhibit respiratory complex I: a common mechanism contributing to their antidiabetic actions? Diabetes 2004;53:1052-9.
337. Stocker DJ, Taylor AJ, Langley RW, Jezior MR, Vigersky RA. A randomized trial of the effects of rosiglitazone and metformin on inflammation and subclinical atherosclerosis in patients with type 2 diabetes. American heart journal 2007;153:445 e1-6.
338. Artwohl M, Furnsinn C, Waldhausl W et al. Thiazolidinediones inhibit proliferation of microvascular and macrovascular cells by a PPARgamma-independent mechanism. Diabetologia 2005;48:586-94.
339. Liu HB, Hu YS, Medcalf RL, Simpson RW, Dear AE. Thiazolidinediones inhibit TNFalpha induction of PAI-1 independent of PPARgamma activation. Biochemical and biophysical research communications 2005;334:30-7.
340. Polikandriotis JA, Mazzella LJ, Rupnow HL, Hart CM. Peroxisome proliferator-activated receptor gamma ligands stimulate endothelial nitric oxide production through distinct peroxisome proliferator-activated receptor gamma-dependent mechanisms. Arteriosclerosis, thrombosis, and vascular biology 2005;25:1810-6.
341. Sasaki M, Jordan P, Welbourne T et al. Troglitazone, a PPAR-gamma activator prevents endothelial cell adhesion molecule expression and lymphocyte adhesion mediated by TNF-alpha. BMC physiology 2005;5:3.
342. Ceolotto G, Gallo A, Papparella I et al. Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD(P)H oxidase via AMPK-dependent mechanism. Arteriosclerosis, thrombosis, and vascular biology 2007;27:2627-33.
343. Forst T, Karagiannis E, Lubben G et al. Pleiotrophic and anti-inflammatory effects of pioglitazone precede the metabolic activity in type 2 diabetic patients with coronary artery disease. Atherosclerosis 2008;197:311-7.
344. Schneider CA, Ferrannini E, Defronzo R, Schernthaner G, Yates J, Erdmann E. Effect of pioglitazone on cardiovascular outcome in diabetes and chronic kidney disease. J Am Soc Nephrol 2008;19:182-7.
345. Home PD, Pocock SJ, Beck-Nielsen H et al. Rosiglitazone evaluated for cardiovascular outcomes--an interim analysis. N Engl J Med 2007;357:28-38.
346. Laufs U, Gertz K, Dirnagl U, Bohm M, Nickenig G, Endres M. Rosuvastatin, a new HMG-CoA reductase inhibitor, upregulates endothelial nitric oxide synthase and protects from ischemic stroke in mice. Brain research 2002;942:23-30.
347. Laufs U, Gertz K, Huang P et al. Atorvastatin upregulates type III nitric oxide synthase in thrombocytes, decreases platelet activation, and protects from

cerebral ischemia in normocholesterolemic mice. Stroke; a journal of cerebral circulation 2000;31:2442-9.

348. Colhoun HM, Betteridge DJ, Durrington PN et al. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet 2004;364:685-96.
349. Cahoon WD, Jr., Crouch MA. Preprocedural statin therapy in percutaneous coronary intervention. The Annals of pharmacotherapy 2007;41:1687-93.
350. Sun W, Lee TS, Zhu M et al. Statins activate AMP-activated protein kinase in vitro and in vivo. Circulation 2006;114:2655-62.
351. Xenos ES, Stevens SL, Freeman MB, Cassada DC, Goldman MH. Nitric oxide mediates the effect of fluvastatin on intercellular adhesion molecule-1 and platelet endothelial cell adhesion molecule-1 expression on human endothelial cells. Annals of vascular surgery 2005;19:386-92.
352. Laufs U, Endres M, Custodis F et al. Suppression of endothelial nitric oxide production after withdrawal of statin treatment is mediated by negative feedback regulation of rho GTPase gene transcription. Circulation 2000;102:3104-10.
353. Laufs U, Endres M, Stagliano N et al. Neuroprotection mediated by changes in the endothelial actin cytoskeleton. The Journal of clinical investigation 2000;106:15-24.
354. Wenzel P, Daiber A, Oelze M et al. Mechanisms underlying recoupling of eNOS by HMG-CoA reductase inhibition in a rat model of streptozotocin-induced diabetes mellitus. Atherosclerosis 2008;198:65-76.
355. Nissen SE, Tuzcu EM, Schoenhagen P et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. The New England journal of medicine 2005;352:29-38.
356. Cool B, Zinker B, Chiou W et al. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab 2006;3:403-16.
357. Andersson U, Filipsson K, Abbott CR et al. AMP-activated protein kinase plays a role in the control of food intake. The Journal of biological chemistry 2004;279:12005-8.
358. McCullough LD, Zeng Z, Li H, Landree LE, McFadden J, Ronnett GV. Pharmacological inhibition of AMP-activated protein kinase provides neuroprotection in stroke. J Biol Chem 2005;280:20493-502.
359. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. Circulation 2002;106:679-84.
360. Parulkar AA, Pendergrass ML, Granda-Ayala R, Lee TR, Fonseca VA. Nonhypoglycemic effects of thiazolidinediones. Ann Intern Med 2001;134:61-71.
361. Yang T, Soodvilai S. Renal and vascular mechanisms of thiazolidinedione-induced fluid retention. PPAR research 2008;2008:943614.